A

THESIS

on the Action of Red Bone-Marrow and Other Substances

on

the Regeneration of the Blood;

and

on the Splenic Anaemia of Infancy.

PART I.

On the Action of Red Bone-Marrow and Other Substances

on

the Regeneration of the Blood.

April 1877.

The subject-matter of the first half of this Thesis is an investigation of some points connected with the effect on the blood of the administration of red bone-marrow. At a time when the actions of many organic extracts have been carefully studied by means of experiments on animals — as examples of this, the thyroid, suprarenal, and thymus, need only be instanced — I expected that the literature bearing on the action of marrow in the treatment of anaemia, both from the clinical point of view, would have been extensive. But this has not been the case. The papers are comparatively few, and many are mere notes or memoranda whose data are so incomplete as to be of little value in enabling one to form any just conclusion as to the facts of the case. It may be thought that I have overlooked papers on the subject, and while this may be so, previous writers have apparently had the same experience, since almost all the articles are distinguished by the paucity of their references.

It will be convenient, <u>first</u>, to consider the clinical evidence bearing on the therapeutic value of bone-marrow, and, <u>second</u>, to discuss the experimental side of the question.

I. C L I N I C A L .

Although, doubtless, in the early days of organo-therapeutics the idea of using red bone-marrow in disorders of the blood must have occurred to many, Goldscheider(1), in 1894, appears to have been the first actually to record its use. Seventeen injections of glycerine extract of marrow got from the bones of calves were given to a patient suffering from pernicious anaemia; no improvement resulted, and the case ended fatally. In a case of leucocythaemia seven injections of marrow and splenic extracts were given; there was slight improvement in the state of the blood, and diminution in the size of the spleen, and the patient's subjective condition was also ameliorated. The effects were, however, but temporary, and were followed by the death of the patient. A month or two later, Dixon Mann(2)recorded the case of a patient suffering from haemophilia with anaemia, as well as three other cases of secondary anaemia, all treated with glycerine extract of calf marrow. In these cases benefit accrued, the rise in corpuscles varying from 300,000 in the course of three weeks to 2,330,000 after nine weeks'treatment. It is expressly stated that no other drugs were employed.

In the same year Billings(3)recorded two cases of pernicious anaemia treated with bone-marrow and arsenic; one of these proved fatal, and in the other, after the marrow had shewn itself useless, arsenic

brought about a cure. Daneforth(4)has reported a case of cure of the same disease by the use of arsenic and marrow. Still in 1894, Forbes(5)treated chlorosis successfully by marrow tabloids; the corpuscles and haemoglobin rose from 2,800,000 and 40 per cent to 3,200,000 and 70 per cent, respectively. The patient is said to have been treated previously by iron without benefit; the details are, however, somewhat meagre.

One of the most important contributions to our knowledge of this subject, and, indeed, that which has been the principal means of bringing the remedy before the profession, is that of Fraser(6). The case was one of pernicious anaemia, and the treatment adopted is divisible into eight periods. It is unnecessary to describe these in detail: suffice it to say that, while iron, arsenic, salol, and splenic extract, were given at varying times along with bone-marrow, it was only after the last-named was administered that improvement began, and that the improvement continued uninterruptedly while the marrow was taken, quite irrespectively of changes in the other medicinal treatment. On admission to hospital the red corpuscles of this patient were as low as from 1,860,000 to 1,400,000 and the haemoglobin 28 to 30 per cent; under treatment by iron they fell still lower, but, beginning to increase as bone-marrow was given, reached a practically normal figure in about twenty weeks, and remained at this level as long as the patient was under observation. It is

right also to say that, along with the improved condition of the blood, there was a corresponding gain in health and strength: the patient seems, in fact, to have been to all intents and purposes cured.

Drummond(7)has has also reported a case of pernicious anaemia treated in a somewhat similar manner. The patient was an insane person, and it is stated that, except for the use of the drugs, absolutely no alteration wwas made in her surroundings. In the course of two months, under marrow alone, the corpuscles increased from 1,140,000 to 1,450,000; thereafter iron was given with the result that they increased in a month to 1,850,000. Arsenic was then added, and two and a half months later the corpuscles were 2,500,000. Drummond asserts that "the improvement [in general health] is not sufficiently revealed by the rise in the corpuscles". The patient ultimately died of pneumonia, and the post mortem shewed appearances not incompatible with the existence of pernicious anaemia.

Aliexiew(8) and Brouardel(9) record several cases of malarial cachexia which improved(after other drugs had failed)under the use of from 1½ to 3 ozs. of calf marrow daily. The former of these observers also reparts the cure of several cases of anaemia secondary to purpura, and the improvement of a case of leucocythaemia. Barrs(10)has published a case of pernicious anaemia in which arsenic had been pushed to the extent of producing palsy without influencing the state of the

blood. Bone-marrow was then given(4th June, 1895), the red corpuscles being only 900,000. They steadily rose, and by July 4th had reached 4,000,000, while by August 10th they were up to 5,000,000. Barrs is of opinion that arsenic is of much less value in the relapses of pernicious anaemia than in the early stages; in fact, that "its failure in relapses is quite as striking as its success in the first attack!" In the paper he mentions that he has seen another case of pernicious anaemia, in which marrow entirely failed.

In 1896, Hunt(11)recorded three cases of pernicious anaemia treated with bone-marrow. The first is that of a woman who had previously improved under arsenic; the relapse on account of which she returned to hospital was not alleviated by marrow. After death, evidence of the disease was found. The second patient, a man, had, during the previous four years, been several times in hospital suffering from pernicious anaemia. On the last occasion he was treated with bone-marrow for a fortnight, and then with arsenic for a month, neither drug producing any benefit. In the third case, a woman, marrow was given for a fortnight without result; marked benefit resulted when arsenic was administered. Whait(12)published a case of spleno-myelogenous leucocythaemia treated by marrow. Here(as in Barrs'case) arsenic had been pushed until peripheral neuritis had supervened, and under marrow a very surprising improvement became manifest. In five months a cure was apparently

established — the spleen was of normal size, and the leucocytosis almost absent. The patient then ceased taking marrow, in six months the symptoms recurred, and, a few months later, death occurred from pneumonia.

In addition to the above papers, this subject has apparently been treated of by Watkins(13), Langdon(14), and Cabot(15). To these articles I have been unable to obtain access. Symes(16), discussing the treatment of splenic anaemia, places marrow next to arsenic.

It was my original intention to have tabulated the cases treated with marrow, and to have attempted to draw some conclusions by comparing the figures with those gained from published cases of anaemia, and especially of pernicious anaemia, treated by other reme--dies. I fully expected to have found many records, but such has not been the case, and the absence of the names of German writers from the above summary, has somewhat surprised me. The material at my disposal is too small to admit of any very definite conclusion being drawn, but, speaking generally, the opinion which I have formed, both from reading these cases and from the few patients who have come under my own personal observation, is not such as to lead me to have any very great anticipation of benefitfrom the use of marrowin the majority of cases. At the same time, I am inclined to think that the remedy is to valuable a one to be hastily set aside; it appears certain that

in several cases a temporary, if not a permanent cure has resulted from its use; and that under certain circumstances extract of red bone-marrow has a stimulating effect on the formation of red corpuscles, will, I think, be shewn in the succeeding part of this Thesis.

II. EXPERIMENTAL.

I. Preliminary.

The only paper bearing directly on the subject is one by Danilewsky(17). A pupil of his, Selensky, experimented on healthy and anaemic animals by injecting watery extracts of spleen and bone-marrow under the skin and into the peritoneal cavity. These extracts, whether fresh or boiled, produced a remarkable effect on the number of the red corpuscles. In healthy dogs, the injection of 10 CC. splenic extract caused a rise of from a quarter to three-quarters of a million, and a corresponding, though less distinct increase in the haemoglobin. In dogs which had been fed for some time on a diet poor in albumin, and in whom there had consequently been a decrease in the corpuscular elements of the blood, splenic injections produced a yet more marked result, the rise being in some cases as great as one million, or even more. Injections of red marrow had very similar effects.

In addition to making observations on dogs, rabbits were also employed. The following will serve as examples of Danilewsky's experiments:-

Experiment 1. (Op. cit. p. 266) Rabbit.

Date.	Red corpuscles.	Haemoglobin.
March 22.	5,815,000	13.1
23.	5,805,000	13.7
Intra-peritoneal injection of 10 CC. fresh splenic extract. 24.	6,040,000	13
26.	6,335,000	13.4

March 27.	6,390,000	13.2
28.	6,345,000	13
30.	6,320,000	12.9
April 3.	6,350,000	13.6
4.	-	13.8
12.	6,355,000	14.4

Experiment 2. (Op. cit. p.267) Dog; fed on rice only. Number of erythrocytes diminished.

Date.	Red corpuscles.	Haemoglobin.
Aug. 8.	3,980,000	13.5
10.	3,985,000	13.8
Intra-peritoneal injection of 20 CC fresh splenic extract. 11.	4,265,000	15.2(!)
12.	4,275,000	15.6
14.	4,610,000	15.5
16.	4,690,000	-
28.	4,620,000	-

Similar results were obtained when boiled splenic extract was used. The only observations on the action of bone-marrow quoted by Danilewsky are the two following experiments:-

Experiment 1. (Op. cit. p.273) Dog; rice diet.

Date.	Red corpuscles.	Haemoglobin.
Oct. 15.	4,427,000	-
16.	4,340,000	18
Intra-peritoneal injection of 20 CC. fresh marrow extract. 17.	4,890,000	19.7
18.	5,087,000	20.3
20.	5,333,000	21
21.	5,287,000	20.4
22.	5,150,000	20.2

The second experiment yields an almost identical result

Experiment 2. (ibid.). Dog; rice diet.

Date.	Red corpuscles.	Haemoglobin.
Oct. 15.	4,007,000	-
16.	4,030,000	18.5
Intra-peritoneal injection of 20 CC. fresh marrow extract. 18.	4,862,000	20.7
19.	5,185,000	20.8
20.	5,014,000	19.6
21.	4,680,000	18.4

In order to estimate the action of a substance in stimulating the production of red corpuscles, it was thought advisable to use animals which were in a state of anaemia. For, so far as clinical experience goes, it is impossible(except perhaps by residence at high altitudes)to increase the red corpuscles of a healthy person above normal limits. On the other hand, certain drugs have the power of raising the corpuscles to the normal level when they have fallen below it. Stockman and Greig(18)found that in the case of young rabbits to which small doses of arsenic had been given over a considerable period of time, there was no increase in the red corpuscles, notwithstanding evidence of great activity of the bone-marrow. In judging of the effects of injections of marrow, therefore, anaemic animals were generally employed.

The first question, then, which arose was how to produce an artificial anaemia which should most closely

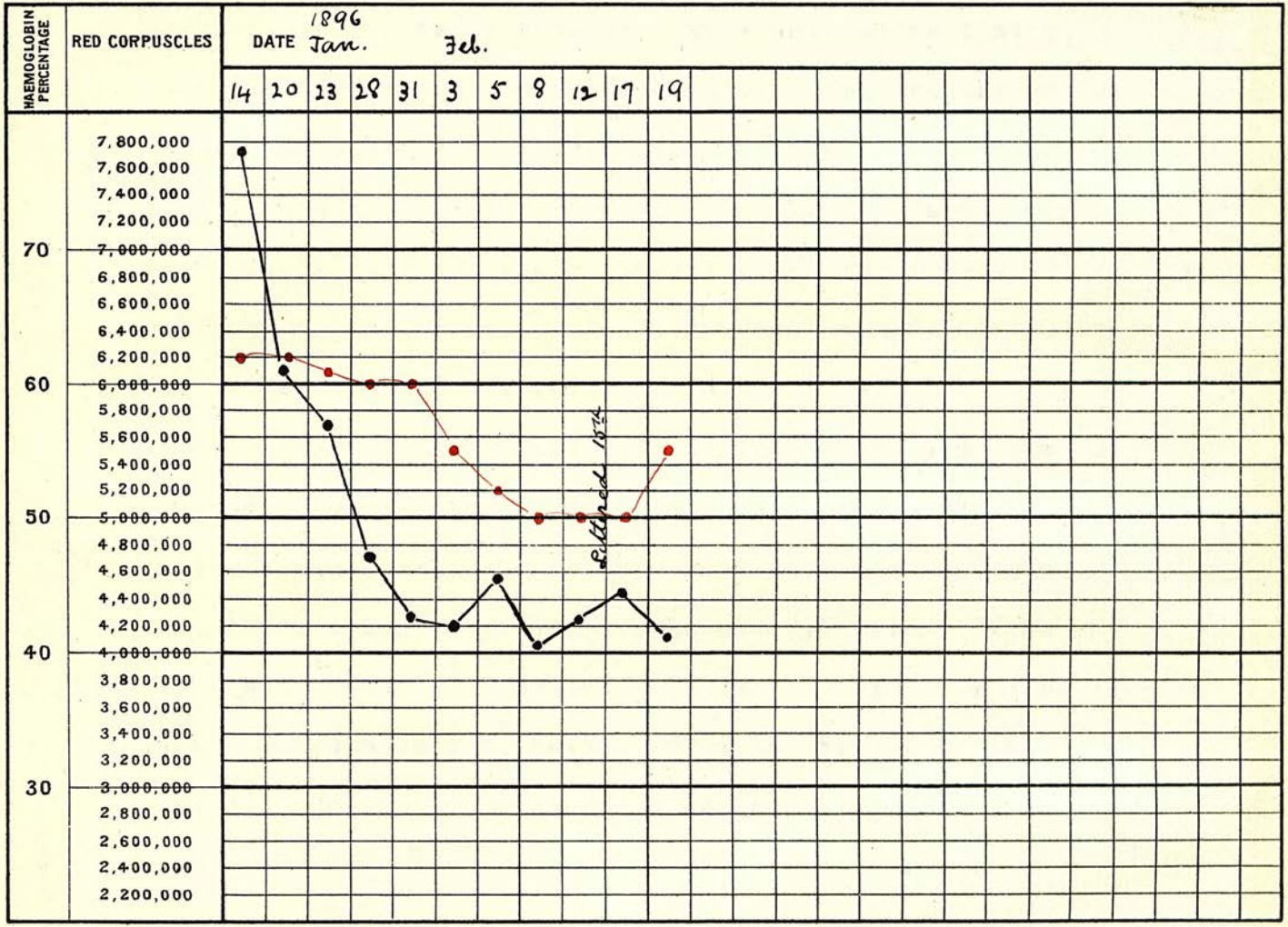
resemble that met with clinically. Obviously, three methods were possible: repeated bleedings, the use of some such substance as pyrogallic acid, which would cause excessive haemolysis, or the use of a diet poor in the constituents needed for blood formation. The objections to the first and second methods seemed sufficiently clear. By neither of them is it possible to produce a steady diminution in the corpuscles, and the constant regenerative action of nature would seriously interfere with attempts to estimate the effects of artificial stimuli

II. Methods.

Before proceeding to the effects of diet, it is perhaps worth while to describe the methods employed. Rabbits were the animals selected for experiment; permission was also obtained to use a few dogs, but they were soon found to be much less easy to manage than the former.

A point in which Danilewsky's experiments seemed inadequate was the comparatively small number of estimations of the corpuscles made in each animal. It appeared desirable that, before deciding that a result was directly due to an injection, the animal should have been kept so long under observation as to allow one to

Experiment 1. Anaemia of pregnancy.



conclude that the number of corpuscles was pursuing a steady course, and not varying from day to day. And experience proved the correctness of this. For it not infrequently happened that, after estimating the corpuscles of a rabbit daily during ten days or a fortnight, one found that for some reason the variations from time to time were very considerable — sufficient, certainly, to vitiate any inference drawn as to the action of a drug on their number. One must, therefore, be certain, that in the animal under observation the corpuscles are either remaining at an even level, or at all events pursuing a definite downward course.

The best rabbits to employ are males of about two months old; these are most easily affected by diet, and probably their haemopoietic organs respond more readily to stimuli than do those of fully-grown animals. Adult female rabbits should not be used unless it is certain that they are not pregnant; the accompanying table and chart shew the course of the corpuscles during pregnancy—— a process which of course renders it futile to make any observation on the effect of injections on them

EXPERIMENT I. Adult female rabbit. During observation corpuscles fell steadily, and after a month the animal littered.

Date.	Red corpuscles.	Haemoglobin.	Weight (grms.)
Jan. 14.	7,760,000	62	2455
20.	6,106,000	62.	2510
23.	5,700,000	68	2510

Date.	Red corpuscles.	Haemoglobin.	Weight.
Jan. 28.	4,740,000	60	2500
31.	4,230,000	60	2540
Feb. 3	4,200,000	55	-
5.	4,586,000	52	2570
8.	4,012,000	50	2670
12.	4,260,000	50	2900
15.	Animal littered.		
17.	4,582,000	50	2540
19.	4,186,000	55	2750

A minor point to be attended to in selecting the rabbit, is that one be chosen whose ears are decidedly vascular. When a number of blood-counts have to be made it is a great advantage to have a series of vessels to choose from. These points may appear somewhat trivial, but so much time was wasted during the earlier part of the work by their neglect, that it seems right to mention them here.

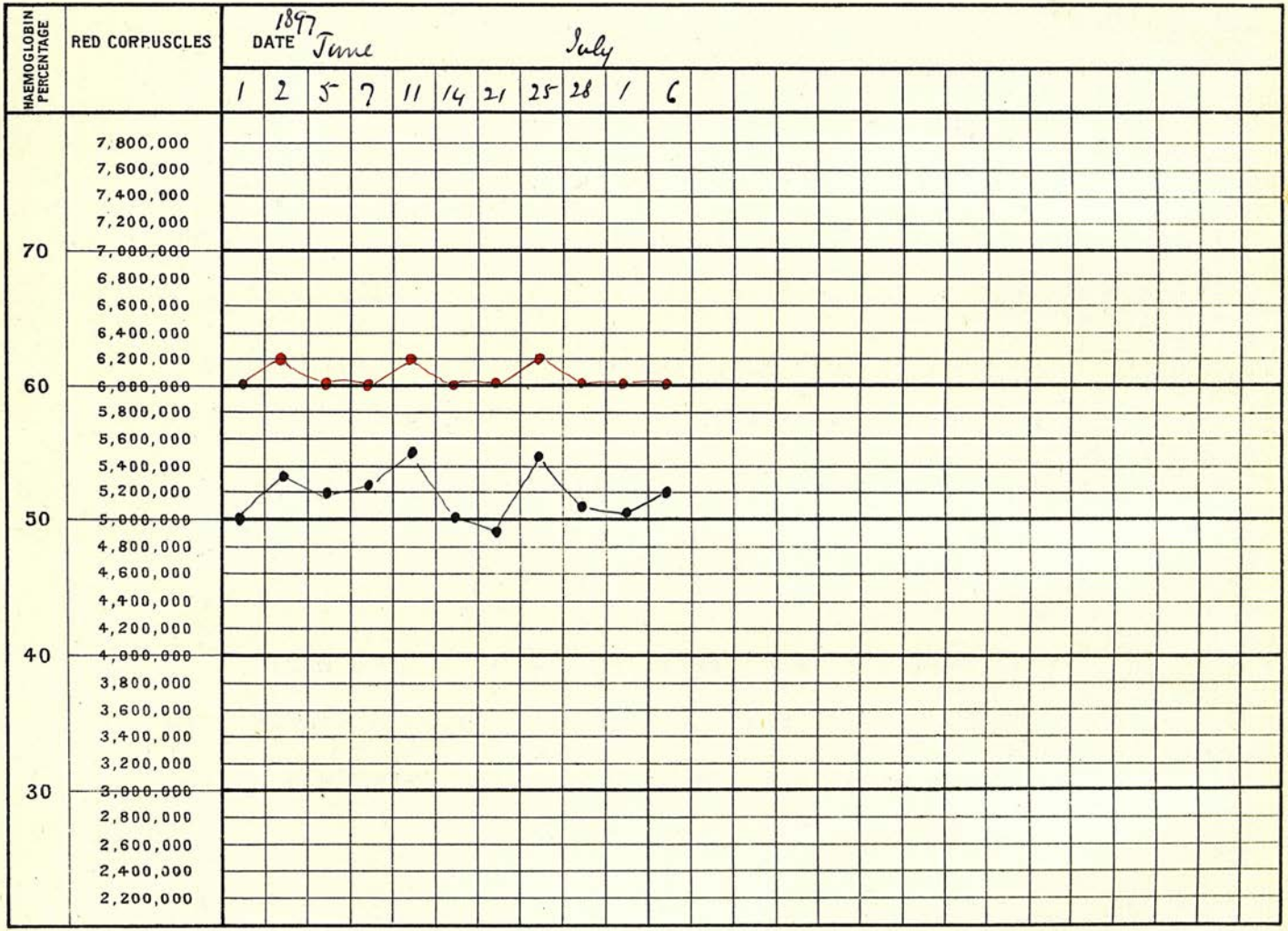
For counting the corpuscles the Thoma-Zeiss instrument was used. The blood was procured from the ears, and in order to obtain a drop of sufficient size easily, it was found well to wait until the vessels were in a state of vaso-dilatation. It has appeared to me, though I have no very definite figures to prove it, that more uniformity is got when the blood is taken from fully dilated vessels, than when it is taken indiscriminately during dilatation and contraction. When a number of blood-counts in the same animal are contemplated, it is well to begin as

near to the tip of the ear as possible; if this be neglected and the blood first drawn from the root of the ear, the vessels are very apt to become partially thrombosed, which makes it difficult to obtain sufficiently large drops of blood later. It is usually better to draw the blood from an arteriole rather than from a vein.

If in the course of any series of observations a blood-count gave an apparently anomalous result, the apparatus was cleaned and a fresh drop of blood taken. If this gave a similar number, the figure was taken as accurate; if a widely differently one, a third estimation was made, which was generally found to be the same as one of the preceding. The average of the two which resembled each other was then taken as approximately correct; this seemed fairer than to average all three and to allow the great error present in one to vitiate the result. As a rule 80 or 100 squares were counted; the dilution was always 1 : 200.

In some(the earlier)estimations of the haemoglobin Gowers' Haemoglobinometer was employed, but latterly I used only Oliver's Tintometer(19). This is a much more convenient and accurate instrument than any other with which I am acquainted; it is certainly superior to v. Fleischl's, and admits of variations of 2 per cent, or even 1 per cent, being quite easily detected.

Experiment 2. Normal diet.



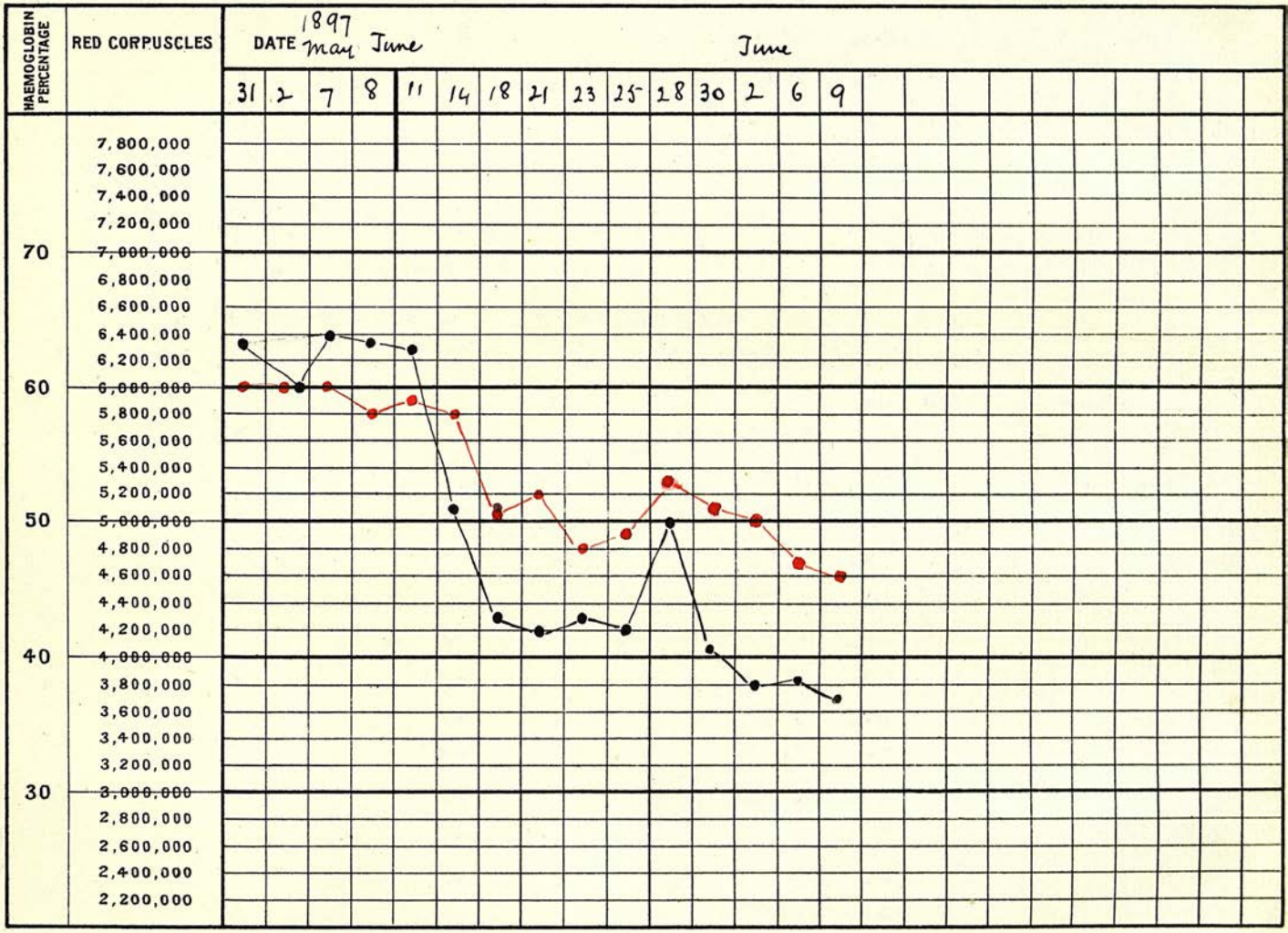
The blood of a rabbit differs somewhat from that of human being in the number of red corpuscles and amount of haemoglobin it contains. A fair average figure is perhaps from 5,800,000 to 6,500,000 corpuscles and from 68 to 72 per cent of haemoglobin.

III. Effects of Diet on the Blood.

To return to the effects of diet on the production of anaemia:- Stockman(20)found that by feeding a dog on a diet from which all iron had been as far as possible removed, but which apparently contained a sufficiency of nitrogenous constituents, the corpuscles not only did not decrease, but actually somewhat increased in number. The haemoglobin value of each corpuscle, however, sank the while from 1 to •66 per cent. As already stated, Danilewsky found that a diet poor in nitrogen and consisting almost entirely of carbohydrate caused a diminution both in the erythrocytes and the haemoglobin.

EXPERIMENTS 2 to 5. Four rabbits from the same litter were taken; one was fed on the ordinary diet (bran and oats, with cabbage or turnip), one on boiled rice, one on boiled rice and egg-albumen, and the fourth on boiled rice and green-stuffs. We were thus enabled to compare the effects of diets poor both in iron and albumin, poor in iron alone, and poor in albumin alone. The results of these observations are seen in the annexed tables and charts — experiments 2 to 5.

Experiment 3. Rice diet from 8th June.



Fall in both red corpuscles and haemoglobin.

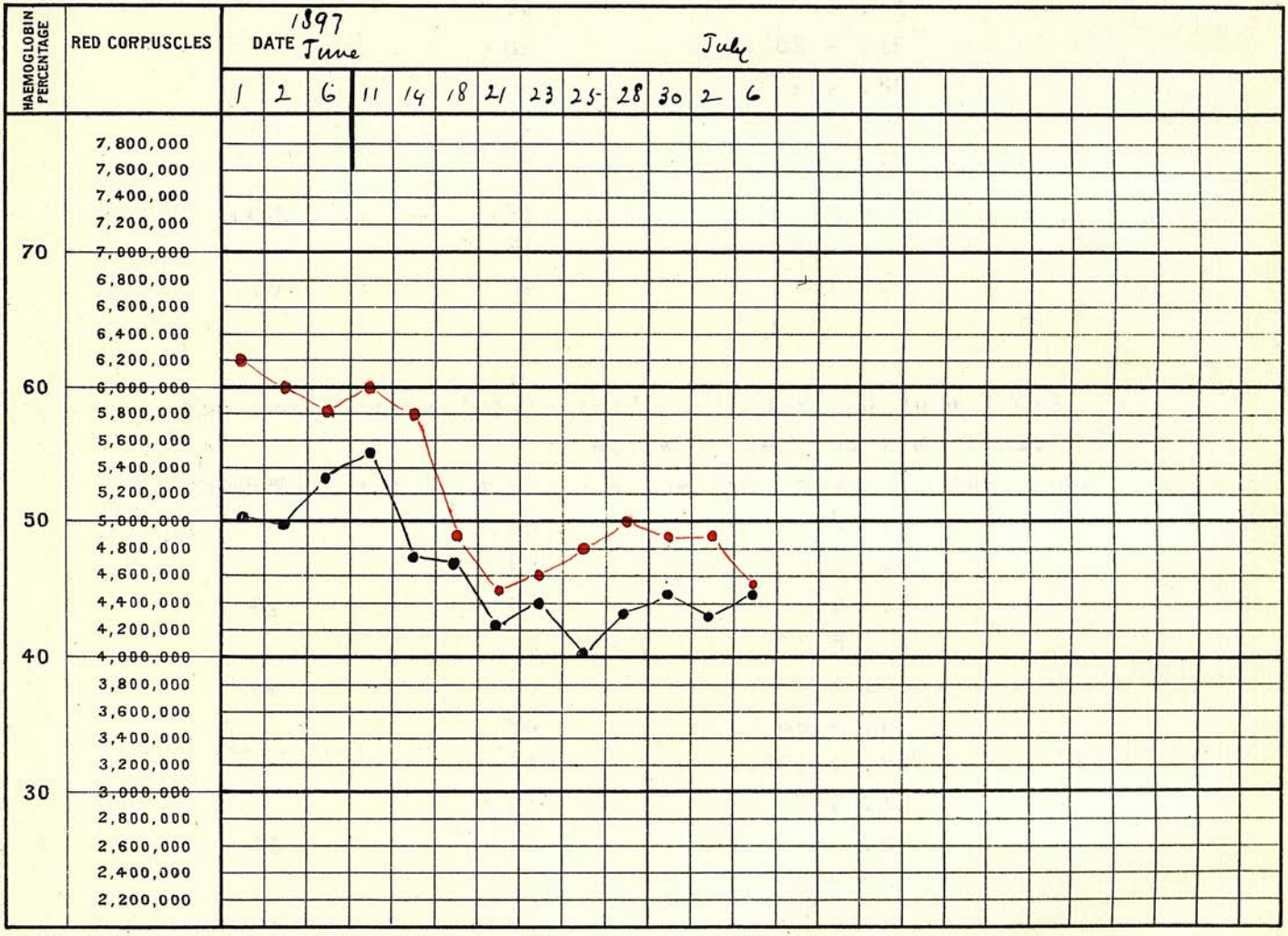
Experiment 2. Rabbit. Ordinary diet.

Date.	Red corpuscles.	Haemoglobin.	Weight.
June 1.	5,000,000	60	340 grm.
2.	5,275,000	62	340
5.	5,200,000	60	340
7.	5,215,000	60	390
11.	5,512,000	62	400
14.	5,000,000	60	460
21.	4,900,000	60	560
25.	5,565,000	62	670
28.	5,100,000	60	690
July 1.	5,050,000	60	690
6.	5,200,000	60	700

Experiment 3. Rabbit. Rice diet from 8th inst.

Date.	Red corpuscles.	Haemoglobin.	Weight.
May 31.	6,350,000	60	270 grm.
June 2.	6,000,000	60	
7.	6,400,000	60	350
8.	6,350,000	58	
11.	6,300,000	59	370
14.	5,025,000	58	
18.	4,300,000	50	400
21.	4,265,000	52	
23.	4,325,000	48	400
24.	4,200,000	49	
28.	5,000,000	53	380
30.	4,050,000	51	
July 2.	3,800,000	47	380
6.	3,830,000	47	
9.	3,700,000	46	370

Experiment 4. Diet of rice and egg-albumen from 7th June.



Fall in haemoglobin relatively greater than in the case of the red corpuscles.

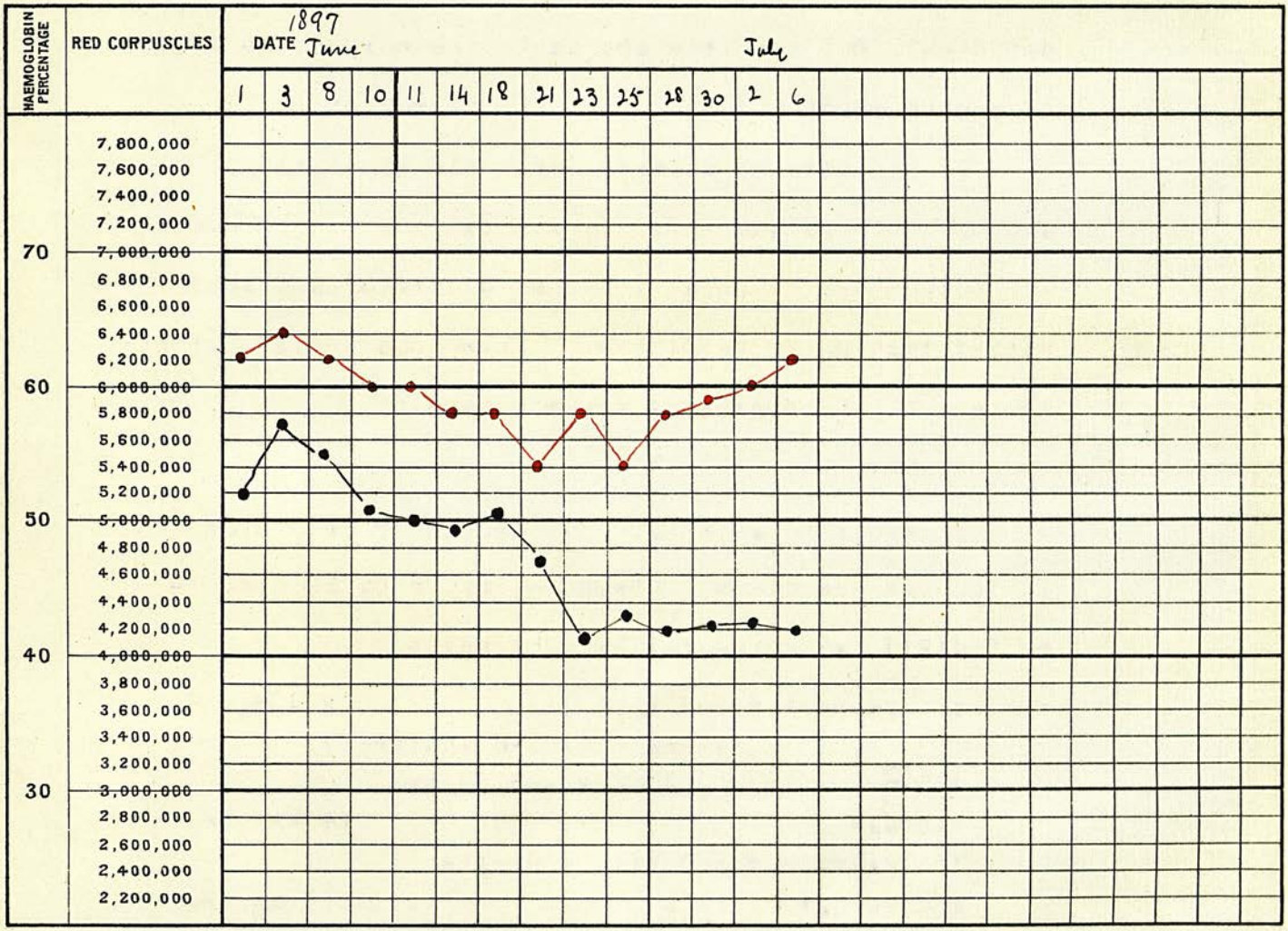
Experiment 4. Rabbit. Diet of boiled rice and egg-albumen from 7th inst. onwards.

Date.	Red corpuscles.	Haemoglobin.	Weight.
June 1.	5,050,000	62	410 gr.
2.	4,925,000	60	
6.	5,350,000	58	460
11.	5,525,000	60	
14.	4,750,000	58	500
18.	4,700,000	49	
21.	4,250,000	45	570
23.	4,400,000	46	
25.	4,060,000	48	620
28.	4,336,000	50	
30.	4,475,000	49	620
July 2.	4,300,000	49	
6.	4,462,000	45	610

Experiment 5. Rabbit. Diet of boiled rice and cabbage from 10th inst. onwards.

Date.	Red corpuscles.	Haemoglobin.	Weight.
June 1.	5,200,000	62	370 gr.
3.	5,670,000	64	
8.	5,500,000	62	430
10.	5,070,000	60	
11.	5,000,000	60	440
14.	4,950,000	58	
18.	5,125,000	58	550
21.	4,700,000	54	
23.	4,150,000	58	520
25.	4,300,000	52	
28.	4,200,000	58	550
30.	4,240,000	59	
July 2.	4,250,000	60	610
6.	4,200,000	62	

Experiment 5. Diet of rice and cabbage from 10th June.



Fall in red corpuscles greater than that of the haemoglobin.

From these tables it will be seen that where both proteid and iron were deficient, the fall in both corpuscles and haemoglobin was marked; where the iron alone was deficient and the proteid abundant the fall in the haemoglobin was relatively greater than in the case of the corpuscles; while where the proteid was deficient, but the iron abundant, the corpuscles fell to a much greater degree than the haemoglobin.

In the case of animals fed entirely on rice, in addition to the quantitative alterations in the blood, the corpuscles eventually become deformed, and rouleaux formation is defective. These changes are proportionate to the degree of anaemia caused.

In order to ascertain the amount of iron present in the various diets, it was estimated by Stockman's method(21), with the following results:-

100 grammes dried rice .....2·4 mg. Fe.
(average of two analyses)
150 grm. mixed bran and oats and 50 gr.
cabbage .....14 mg. Fe.
150 grammes mixed bran and oats
and 100 gr. turnip .....6·7 mg. Fe.

It will be seen that there is a considerable difference in the amounts of iron in the second and third of these analyses. This is apparently due to the large quantity contained in the green vegetable; Bunge (22)shews that these substances contain a high percent-

-age of iron — higher than meat, for example. The quantity of iron got from rice is, however, somewhat larger than that given by Bunge (1·8 mg. per 100 grms.). As white of egg contains at most a mere trace of iron (Bunge, op. cit.), it need not be taken into consideration.

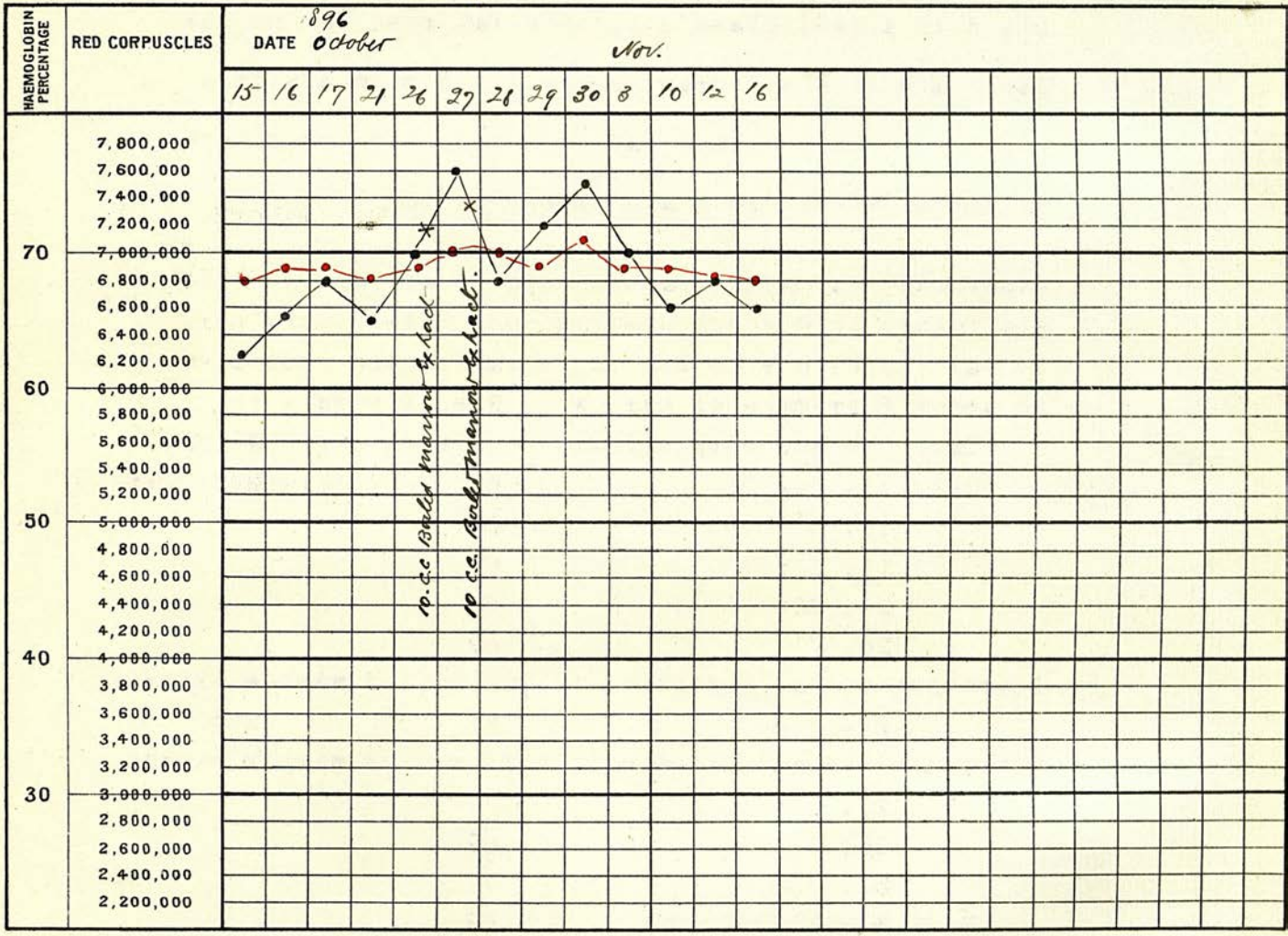
It was found that the ordinary amount of boiled rice eaten by a rabbit in a day was equal to from 70 to 80 grammes of the dry material, so that the difference between the intakes of iron in animals 2. or 5. and 3. or 4. was very considerable. It was thought unnecessary to estimate the nitrogen in the diets.

IV. Action of Bone-marrow on Blood Formation.

In all, eleven experiments were made on the action of red bone-marrow. At first this was obtained at the butcher's, from the long bones of the calf, but both delay and difficulty were experienced in getting a sufficient supply. Eventually, Messrs Burroughs and Wellcome were kind enough to supply me; their sample, I believe, was obtained from the ribs of young animals.

It was found practically impossible to procure fresh marrow with antiseptic precautions; all that could be done was to use it as speedily as possible and to keep the extract on ice. Despite these precautions, however, the injection of one sample (which appeared

Experiment 6. Normal rabbit.



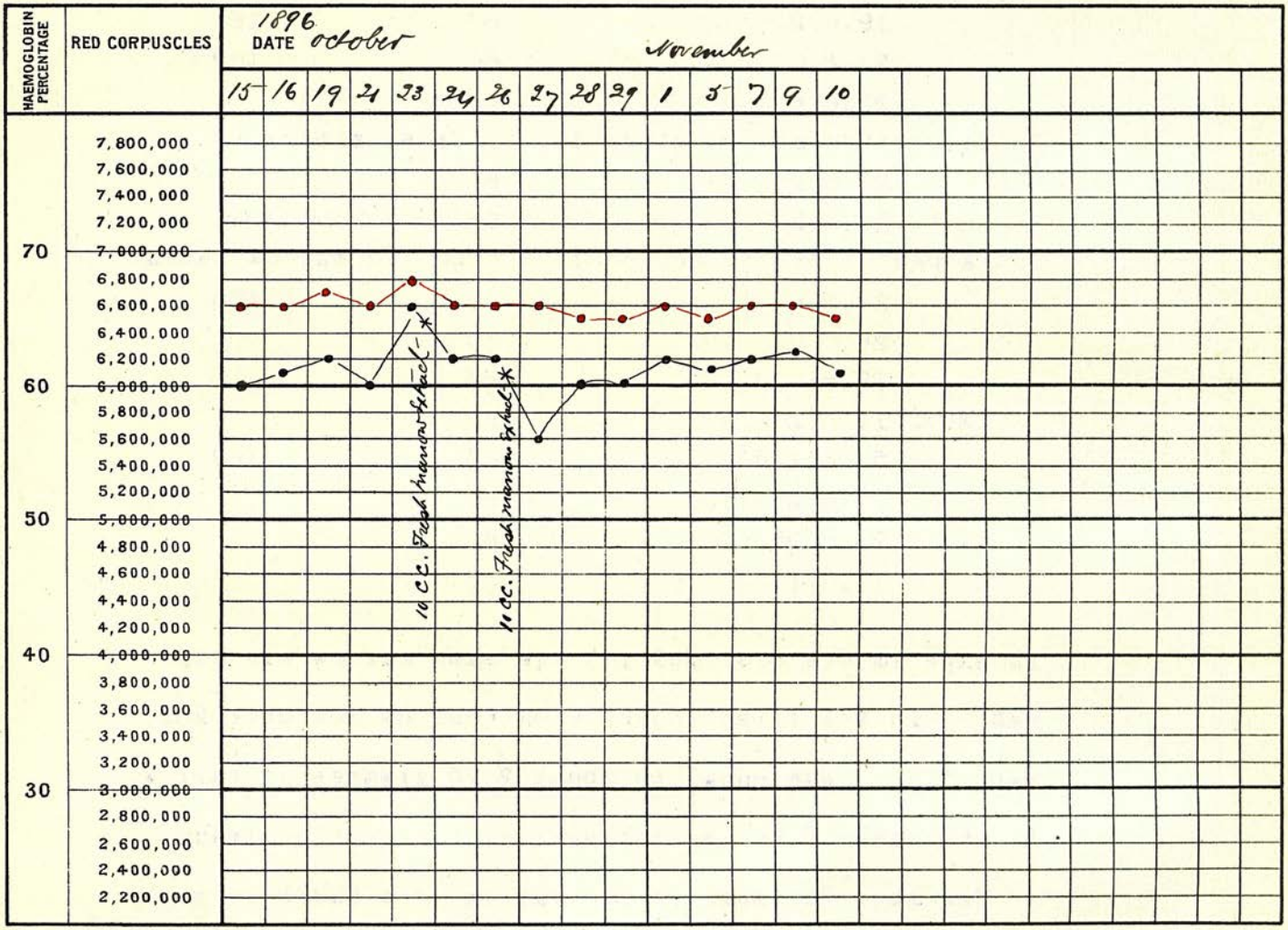
Injections of boiled marrow extract. Negative result.

perfectly fresh)proved rapidly fatal. It was, of course, only when a fresh extract was employed that the risk of contamination proved a drawback. The extracts, whether fresh or boiled, were at first prepared by simply grinding 20 or 30 grammes of marrow with about 50 CC. normal salt solution, allowing to stand in a tall glass until the fat rose to the surface, and then withdrawing the fluid by means of a pipette. The extract was then injected, or boiled, filtered, sterilized, and preserved for use.

Experiment 6. Adult rabbit on ordinary diet; weight stationary throughout observation. Two intra-peritoneal injections of boiled extract, each equivalent to about 6 grammes of marrow. Result negative.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Oct. 15.	6,250,000	68	2080 grm.
16.	6,560,000	69	
17.	6,800,000	69	
21.	6,500,000	68	
26.	7,000,000	69	
Intra-peritoneal injection 10 CC. boiled marrow extract.			
27.	7,600,000	70	2250
Intra-peritoneal injection 10 CC. boiled marrow extract.			
28.	6,800,000	70	
29.	7,200,000	69	
30.	7,500,000	71	
Nov. 3.	7,000,000	69	
10.	6,600,000	69	
12.	6,800,000	68	
16.	6,700,000	68	2080

Experiment 7. Normal rabbit.



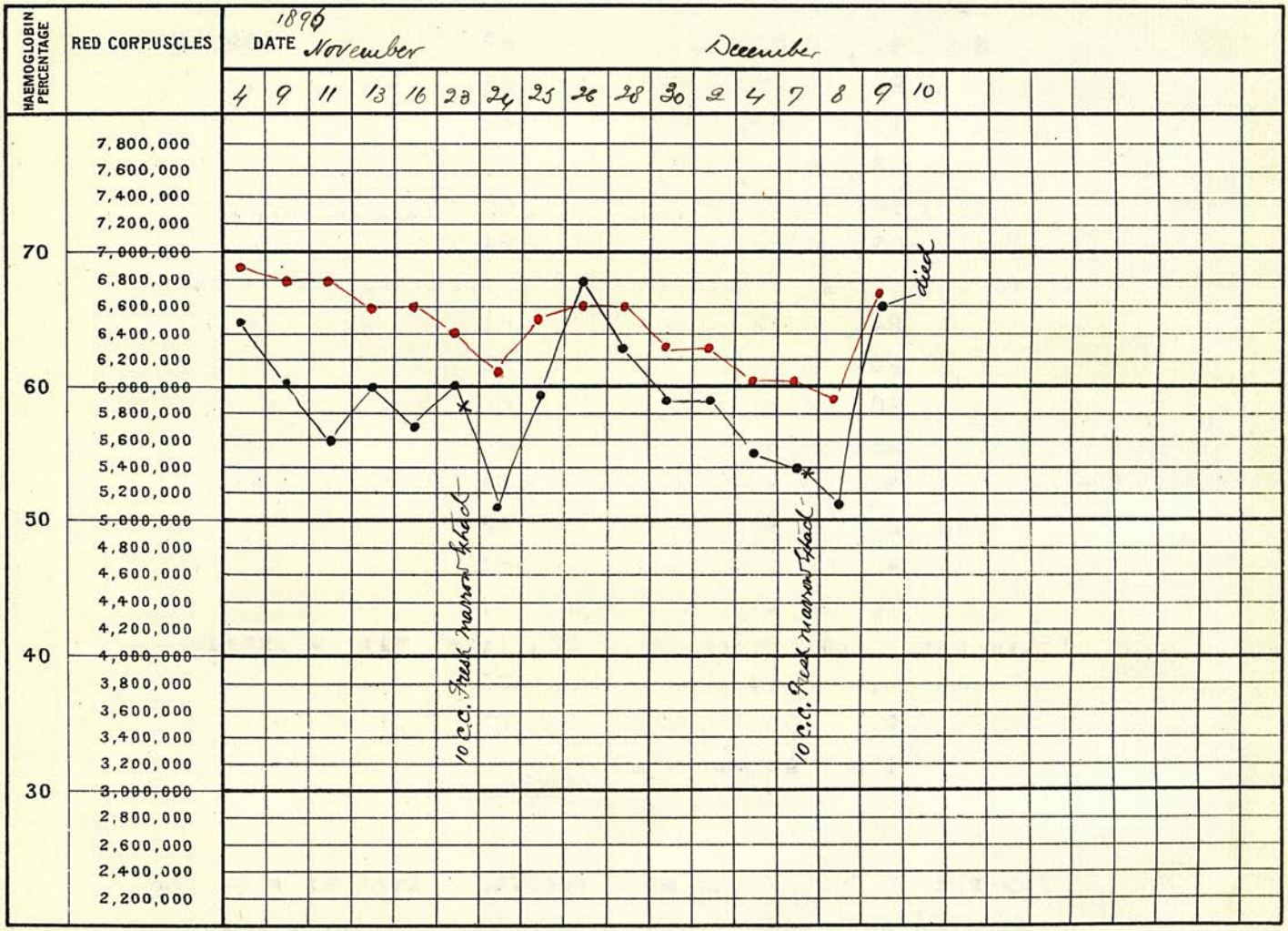
Injections of fresh and boiled marrow-extract. Negative result.

Experiment 7. Adult rabbit on ordinary diet; gain in weight during observation. Two intra-peritoneal injections:- of fresh marrow extract, equivalent to 4·6 grammes marrow, and of boiled extract, equivalent to 6 grammes marrow. Result negative.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Oct. 15.	6,000,000	66	1530 gr.
16.	6,100,000	66	
19.	6,200,000	67	1640
21.	6,000,000	66	1690
23.	6,600,000	68	
Intra-peritoneal injection 10 CC. fresh marrow extract.			
24.	6,200,000	66	
26.	6,200,000	66	
Intra-peritoneal injection 10 CC. boiled marrow extract.			
27.	5,600,000	66	1650
28.	6,000,000	65	
29.	6,000,000	65	
Nov. 1.	6,200,000	66	
5.	6,125,000	65	1690
7.	6,200,000	66	
9.	6,250,000	66	
10.	6,100,000	65	

In experiments 8,9, and 10, the same marrow was employed. In the first injections(those on November 23d) each 10 CC. was equal to about 2·75 grammes of marrow. In the case of the second series of injections(made on December 7th)some of the marrow used had been removed from the bones two days previously. All three animals died after this dose, and on post mortem examination no apparent cause of death could be found. They appear to have died from some form of intoxication.

Experiment 8. Normal rabbit.



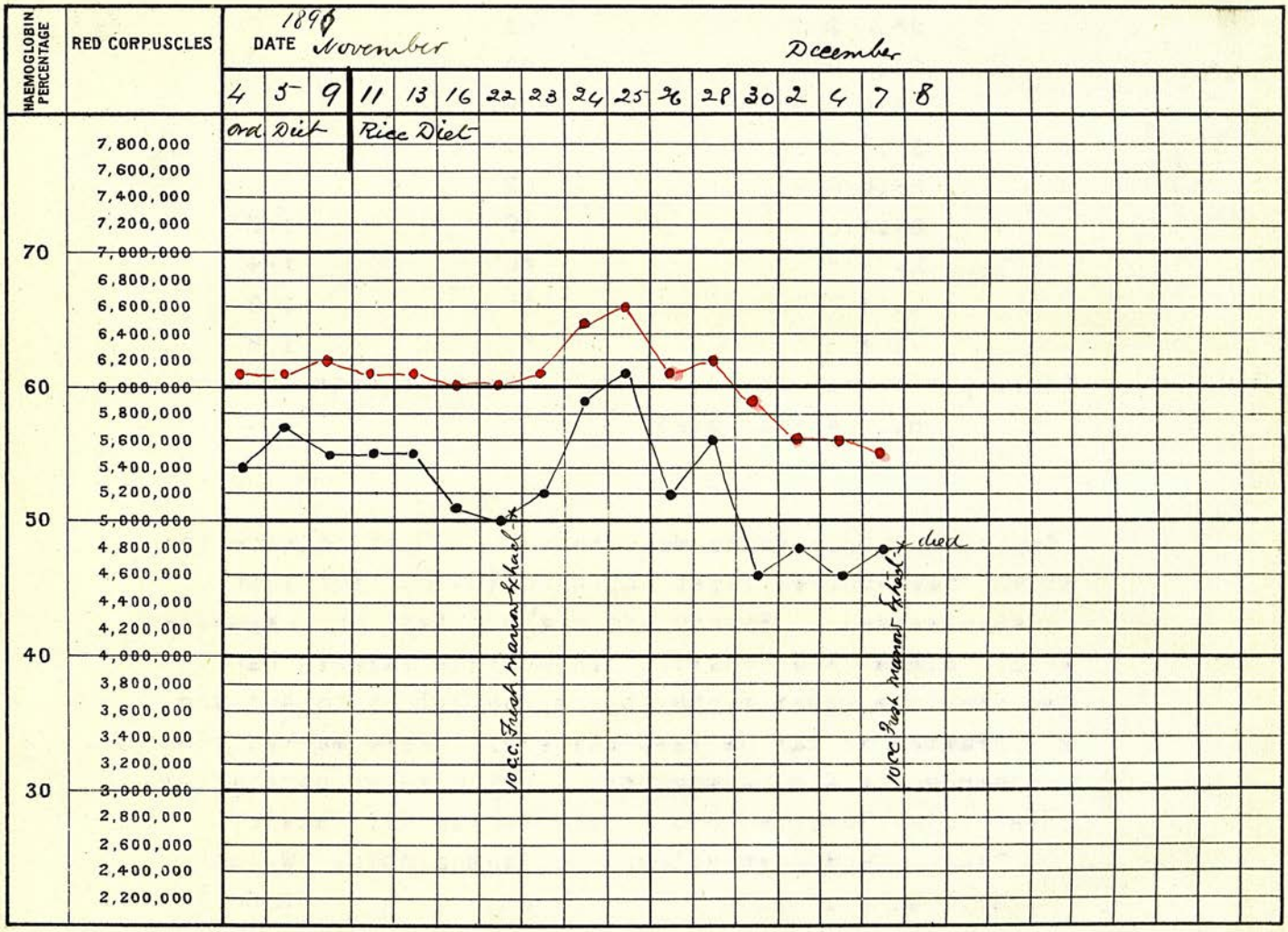
Corpuscles varying. Two injections fresh marrow-extract; second fatal. Result somewhat inconclusive.

Experiment 8. Young male rabbit on ordinary diet; progressive gain in weight. Two intra-peritoneal injections of fresh marrow extract. The second (equal to 1·8 grammes marrow) caused death two days later. A rise of corpuscles took place after each injection, but it will be noticed that the corpuscles had in this animal been inclined to vary considerably. The result is inconclusive.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 4.	6,500,000	69	1520 grm.
9.	6,050,000	68	
11.	5,600,000	68	
13.	6,000,000	66	
16.	5,700,000	66	
23.	6,000,000	64	
Intra-peritoneal injection 10 CC. fresh marrow extract.			
24.	5,120,000	61	1600
25.	5,900,000	65	
26.	6,800,000	66	
28.	6,320,000	66	1870
30.	5,900,000	63	
Dec. 2.	5,900,000	63	
4.	5,500,000	61	
7.	5,400,000	61	
Intra-peritoneal injection 5 CC. fresh marrow extract.			
8.	5,140,000	59	
9.	6,600,000	67	
10.	Animal died.		

Experiment 9. Young male rabbit. Diet of rice for eleven days before first injection (i. e. from 11th inst. onwards). Corpuscles gradually decreasing; gain in weight speady. Two injections of fresh marrow, as in experiment 8.; the second proved fatal. After first injection a distinct rise in corpuscles and haemoglobin lasting for about five days.

Experiment 9. Anaemic rabbit.



Corpuscles falling. Two injections fresh marrow-extract; second fatal. Rise in corpuscles and haemoglobin, lasting five days, after first injection.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 4.	5,400,000	61	1650
5.	5,700,000	61	1660
9.	5,500,000	62	
11.	5,500,000	61	1650
13.	5,500,000	61	
16.	5,100,000	60	1680
22.	5,000,000	60	

Intra-peritoneal injection 10 CC. fresh marrow extract.

23.	5,200,000	61	
24.	5,900,000	65	
25.	6,100,000	66	
26.	5,200,000	61	
28.	5,600,000	62	
30.	4,600,000	59	1720
Dec. 2.	4,800,000	56	1740
4.	4,600,000	56	1720
7.	4,800,000	55	1780

Intra-peritoneal injection 5 CC. fresh marrow extract.

8. Animal died.

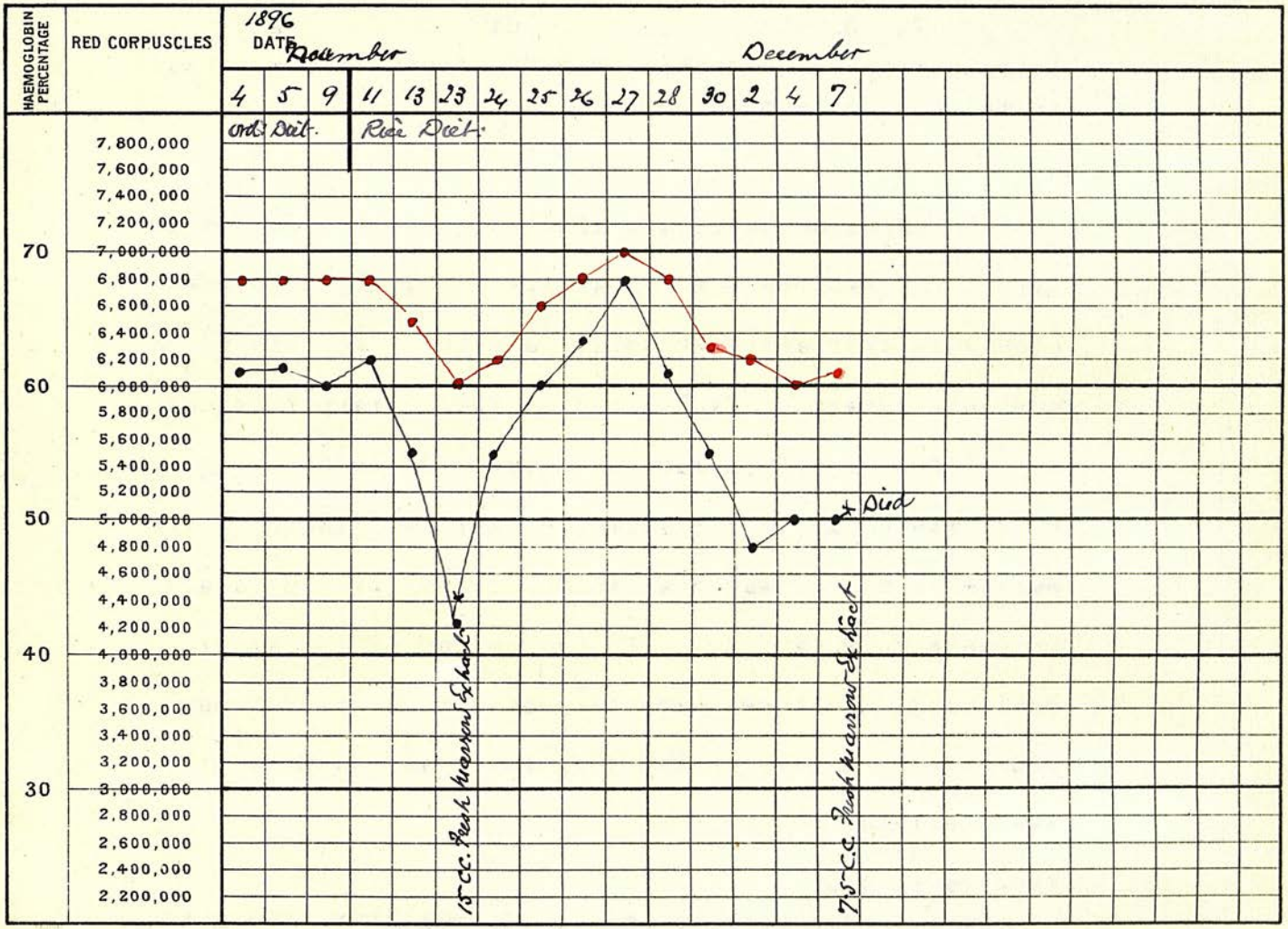
Experiment 10. Young male rabbit. Diet of rice for eleven days before first injection (i. e. from 11th inst. onwards). Steady and decided fall in corpuscles; weight almost stationary. Injections larger than in two previous experiments, being equivalent to 4·1 and 2·7 grammes of marrow respectively. Very marked rise in corpuscles and haemoglobin lasting seven days after first injection; second injection rapidly fatal.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 4.	6,100,000	68	2050
5.	6,120,000	68	
9.	6,000,000	68	
11.	6,200,000	68	
13.	5,500,000	65	
23.	4,250,000	60	2070

Intra-peritoneal injection 15 CC. fresh marrow extract.

24.	5,500,000	62	
25.	6,000,000	66	

Experiment 10. Anaemic rabbit.



Corpuscles falling. Two injections fresh marrow extract; second fatal. Marked rise in corpuscles and haemoglobin, lasting seven days, after first injection.

Experiment 10. (continued).

Date.	Red corpuscles.	Haemoglobin.	Weight.
Dec. 26.	6,330,000	68	2000
27.	6,800,000	70	2050
28.	6,100,000	68	
30.	5,500,000	63	2080
Dec. 2.	4,800,000	62	2050
4.	5,000,000	60	2050
7.	5,000,000	61	2100

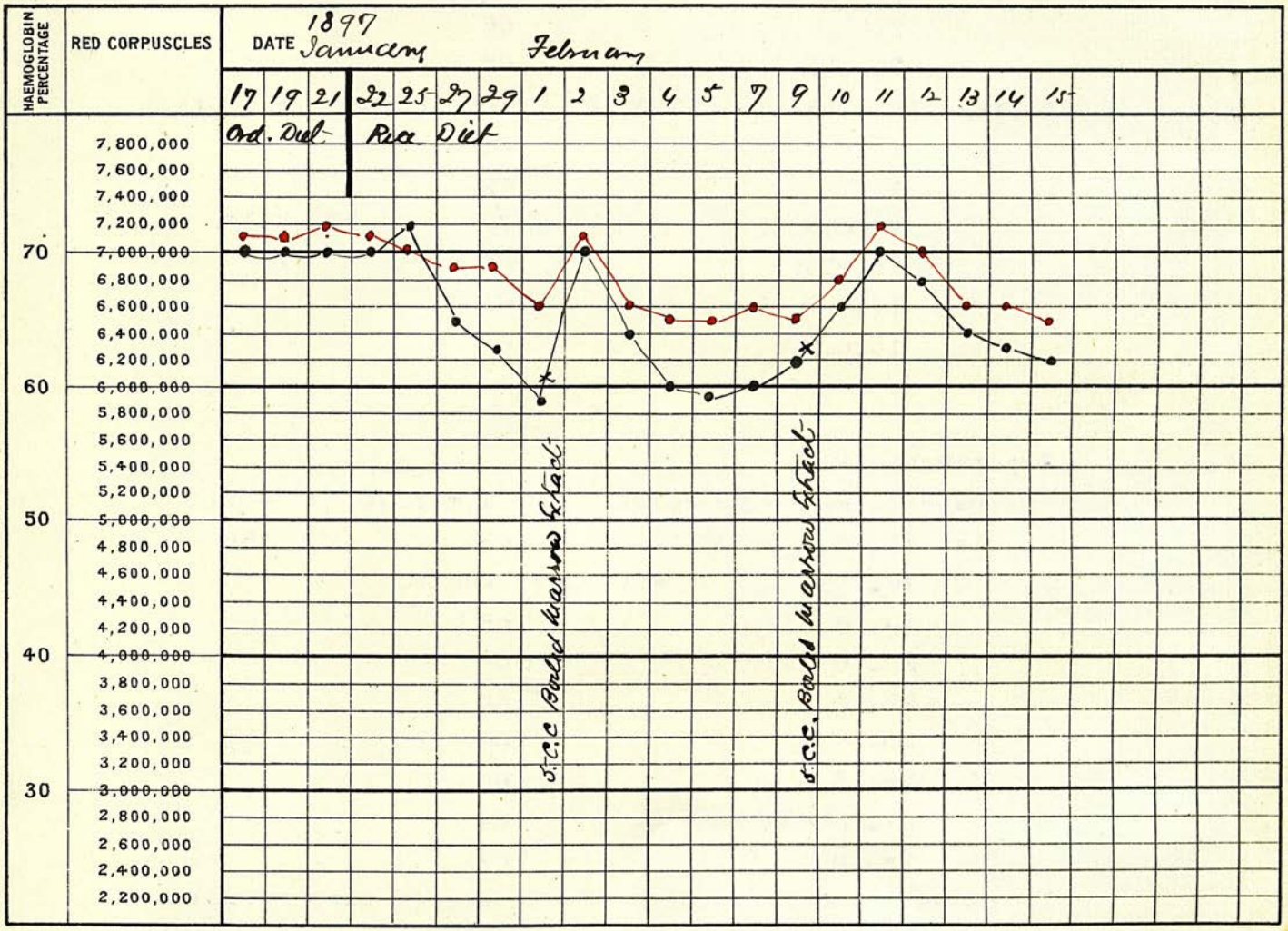
Intra-peritoneal injection 7·5 CC. fresh marrow extract. Animal died on same evening.

In experiments 11 and 12 the conditions were similar. The rabbits were on a rice diet; their corpuscles were decreasing steadily, and their weights almost stationary. Each received two intra-peritoneal injections of boiled marrow extract, of which every 5 CC. was equal to 1·8 gramme of marrow. (Note. The marrow used here was that which, fresh, proved fatal in cases 8, 9, and 10. It had no bad effect after boiling.) In these two observations the result was positive — a rise in the corpuscles and haemoglobin was observed.

Experiment 11.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Jan. 17.	7,000,000	71	2280 gr.
19.	7,000,000	71	
21.	7,000,000	72 Rice diet begun.	
22.	7,000,000	71	
25.	7,200,000	70	
27.	6,500,000	69	
29.	6,300,000	69	2200

Experiment 11. Anaemic rabbit.



Corpuscles falling. Two injections boiled marrow-extract, each followed by rise in corpuscles and haemoglobin.

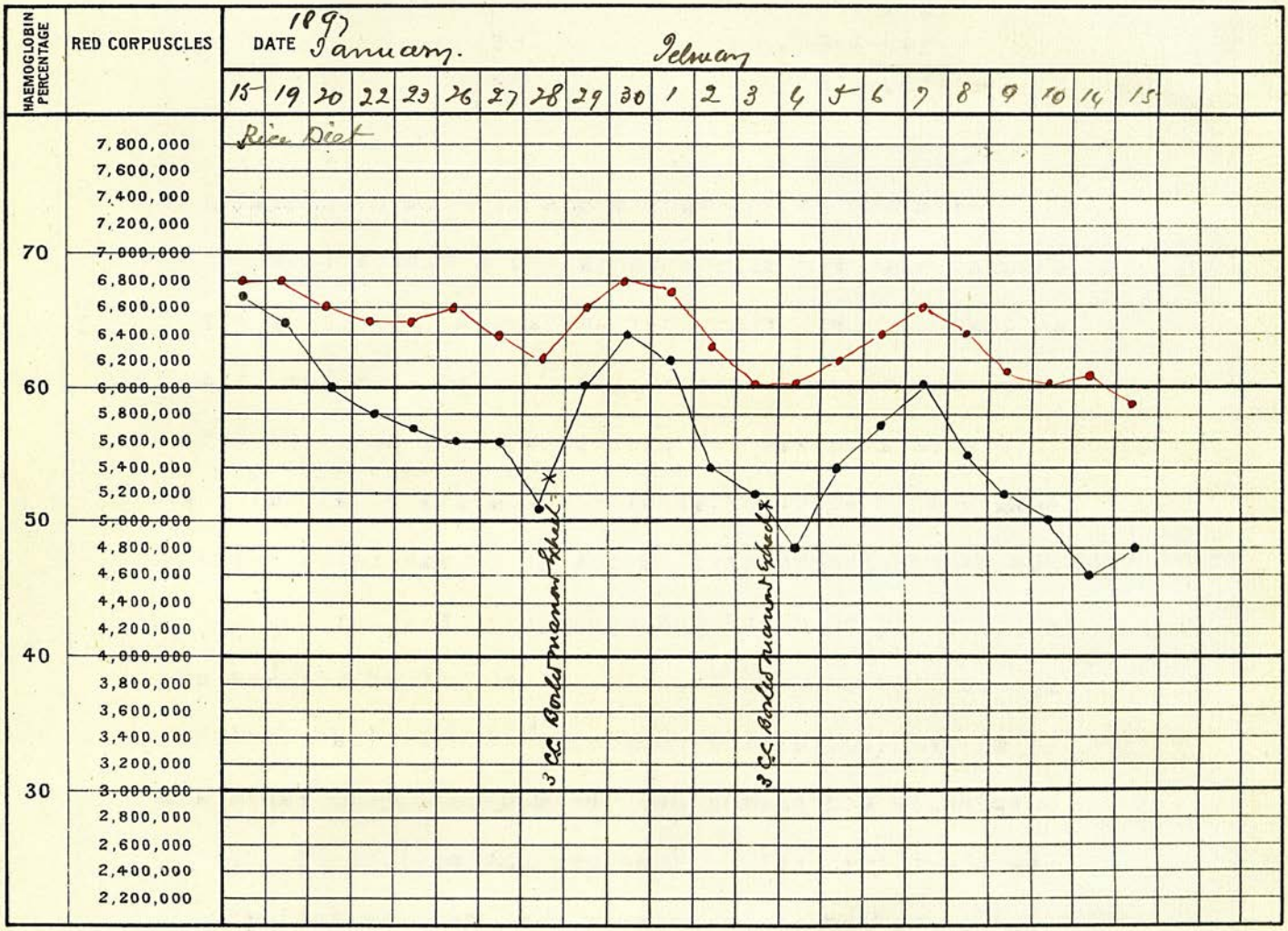
Experiment 11. (continued).

Date.	Red corpuscles.	Haemoglobin.	Weight.
Feb. 1.	5,900,000	66	
Intra-peritoneal injection 5 CC boiled marrow extract.			
2.	7,000,000	71	2200
3.	6,400,000	66	
4.	6,000,000	65	
5.	5,950,000	65	
7.	6,000,000	66	
9.	6,200,000	65	
Intra-peritoneal injection 5 CC. boiled marrow extract.			
10.	6,600,000	68	
11.	7,000,000	72	
12.	6,800,000	70	
13.	6,400,000	66	2200
14.	6,300,000	66	
15.	6,200,000	65	

Experiment 12.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Jan. 15.	6,700,000	68	1860
16.	Rice diet begun.		
19.	6,500,000	68	
20.	6,000,000	66	
22.	5,800,000	65	
23.	5,600,000	66	
26.	5,600,000	66	
27.	5,600,000	64	
28.	5,100,000	62	
Intra-peritoneal injection 3 CC. boiled marrow extract.			
29.	6,000,000	66	
30.	6,400,000	68	
Feb. 1.	6,100,000	67	
2.	5,400,000	63	1850
3.	5,100,000	60	
Intra-peritoneal injection 5 CC. boiled marrow extract.			
4.	4,800,000	60	1900
5.	5,400,000	62	

Experiment 12. Anaemic rabbit.



Corpuscles falling. Two injections boiled marrow-extract, each followed by rise in corpuscles and haemoglobin.

Experiment 12. (continued).

Date.	Red corpuscles.	Haemoglobin.	Weight.
Feb. 6.	5,750,000	64	
7.	6,000,000	66	
8.	5,500,000	64	
9.	5,200,000	63	
10.	5,000,000	60	
14.	4,600,000	61	
15.	4,800,000	59	

From the above experiments it seemed tolerably evident that red marrow contained a substance which, at least under certain circumstances, had the power of stimulating blood-formation. And, further, that this substance was not precipitated by boiling, but remained in solution after the coagula of albumin, etc., had been filtered off; and that it was not a ferment, which would be destroyed by heat. Neither did it appear likely to me that the stimulant action was due to an iron-containing compound; the increase in the corpuscles and haemoglobin seemed to be too rapid and transient for that. However, estimations of the iron contained in the marrow were made as follows:-

100 grammes of red marrow was ground up with saline solution, and(as in preparing an extract for injection) the fats separated as far as possible. The solids were then coagulated by boiling, and the extract filtered.

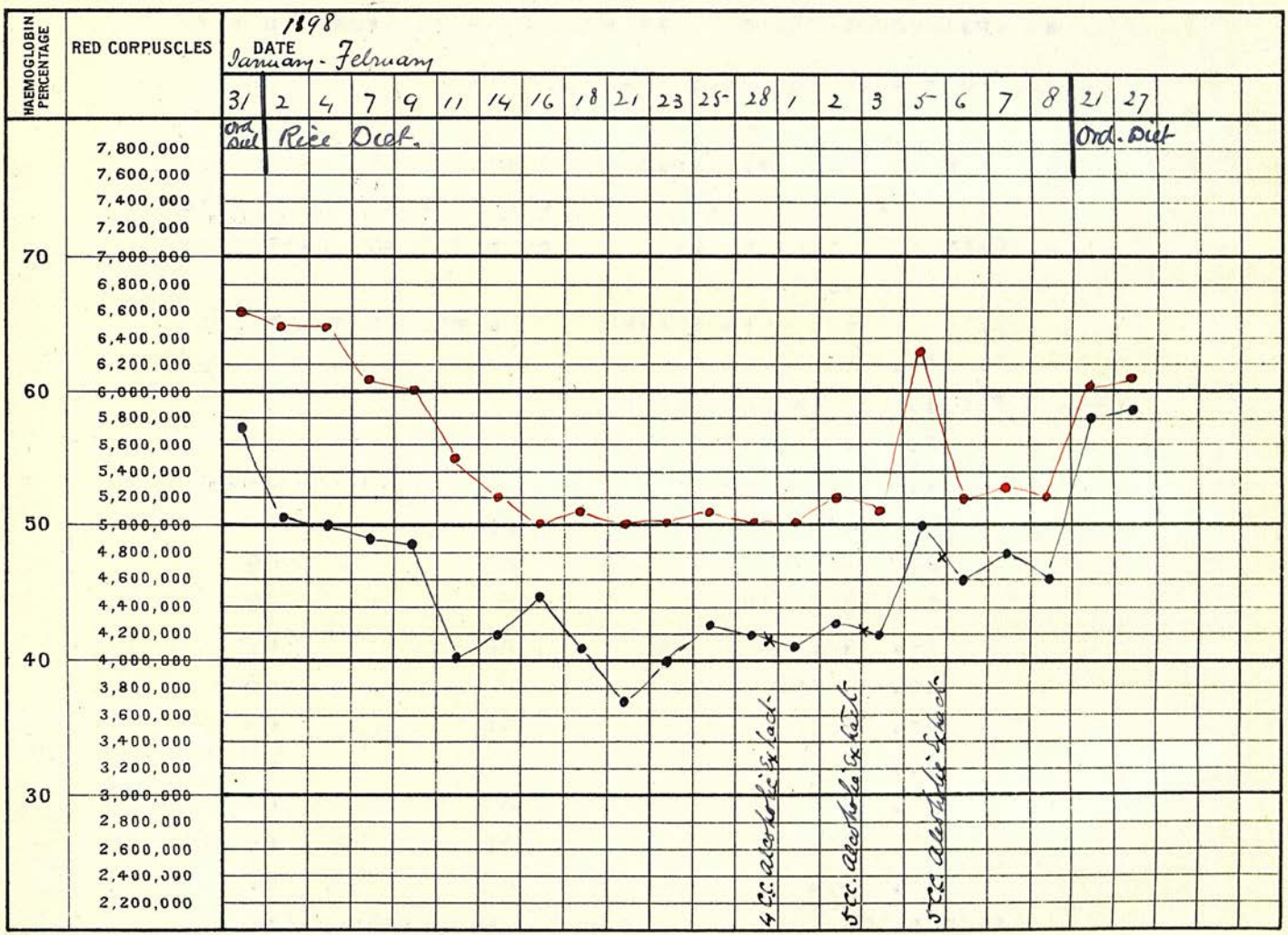
The separated fats, etc. yielded . . .	3 mg. Fe.
Solids precipitated by heat yielded .	3·5 mg. Fe.
Filtrate yielded	2·2 mg. Fe.
Total	8·7 mg. Fe.

By a curious coincidence this (8·7 mg.) is the exact amount of iron given by Stockman in one of his analyses of 100 grammes of red marrow; in his second he got 7·6 mg. (op. cit.) I am inclined to think that in my estimation there remained in the fats traces of some reducing substance, which I failed to get rid of altogether. But at all events we see that the greater part of the iron is removed from marrow by extraction with saline solution, and that part of it is combined in such a way as not to be precipitated by heat.

In order to arrive at some nearer idea of the active substances or substance in marrow, the following procedure was adopted:-

A quantity of red marrow was allowed to stand for two and a half months under absolute alcohol. The alcohol was then removed, the marrow being washed with fresh alcohol, which was added to that previously used. The marrow was then extracted with ether, so as to remove all fats; the residue was then dried and preserved. The alcoholic extract was evaporated at a temperature of 37 deg. C., and the brownish, slightly sticky residue redissolved in absolute alcohol and normal salt solution, and preserved over sulphuric acid.

Experiment 13. Anaemic rabbit.



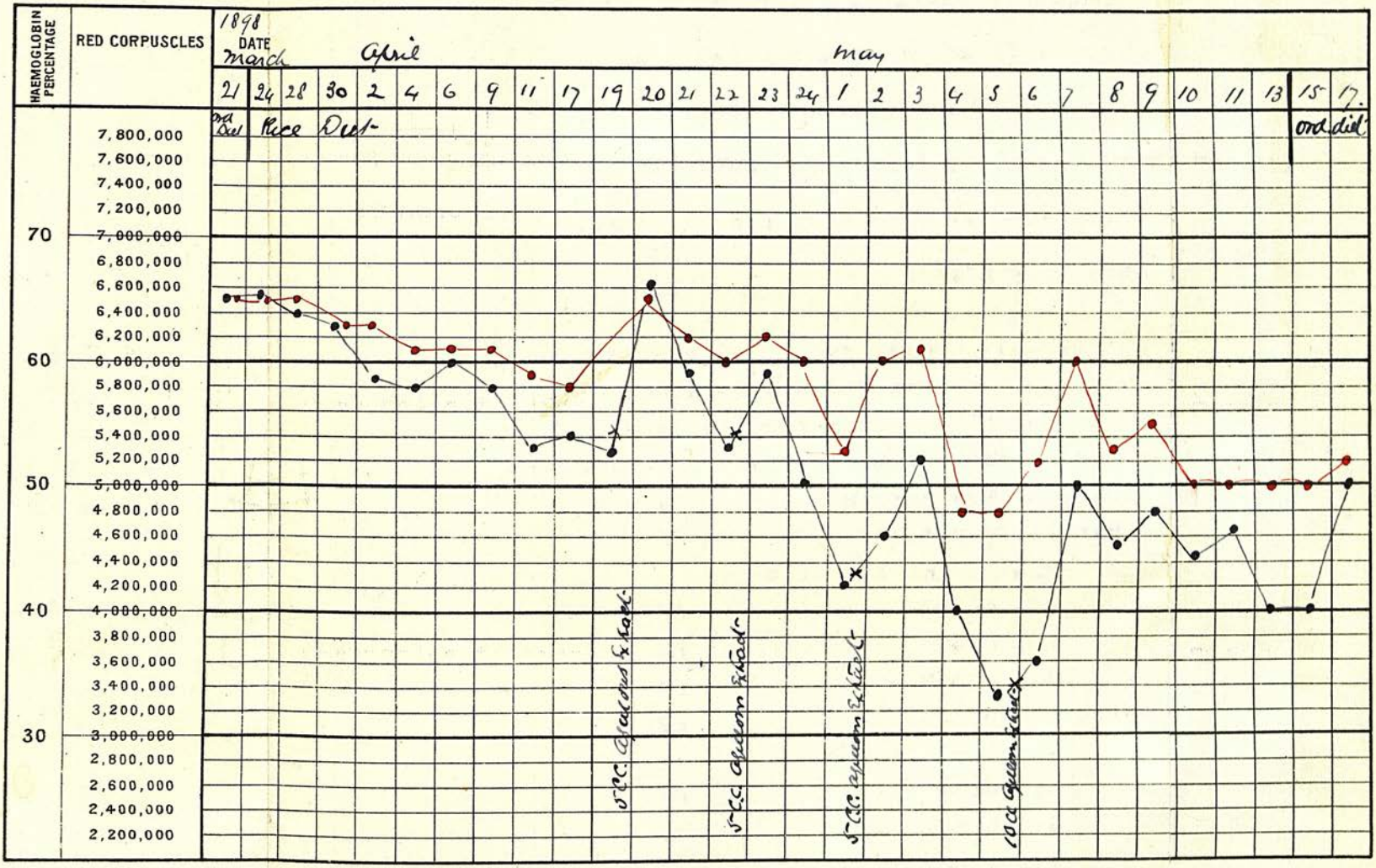
Corpuscles falling. Three injections alcoholic extract of marrow; result negative.

The small quantity of alcoholic extract obtained made it impossible to perform many experiments with it. After driving off the alcohol and salt solution in which it had been dissolved, the extract obtained from 30 grammes of marrow was shaken with 30 CC. normal salt solution, in which it partially dissolved to form an opalescent fluid. As will be seen from experiment 13, no result followed its injection.

Experiment 13. Male rabbit, 1 month old; rice diet. Three intra-peritoneal injections of alcoholic extract of marrow. Steady fall in corpuscles and haemoglobin. Progressive gain in weight.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Jan. 31.	5,750,000	66	500 gr.
Feb. 1.	Rice diet begun.		
2.	5,080,000	65	550
4.	5,000,000	65	480
7.	4,900,000	61	570
9.	4,875,000	60	590
11.	4,000,000	55	620
14.	4,200,000	52	620
16.	4,562,000	50	630
18.	4,100,000	51	630
21.	3,700,000	50	600
23.	4,000,000	50	660
25.	4,175,000	51	680
28.	4,200,000	50	690
Intra-peritoneal injection 4 CC. alcoholic extract.			
Mar. 1.	4,100,000	50	700
2.	4,362,000	52	
Subcutaneous injection 5 CC. alcoholic extract.			
3.	4,100,000	51	670
5.	5,000,000	63	
Subcutaneous injection 5 CC. alcoholic extract.			
6.	4,600,000	52	
7.	4,800,000	53	

Experiment 14. Anaemic rabbit.



Corpuscles falling. Four injections aqueous extract of marrow, each followed by rise in corpuscles and haemoglobin. These rises, however, do not check the general progressive course of the anaemia.

Experiment 13. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Mar. 8.	4,625,000	52	
10.	Ordinary diet.		
21.	5,800,000	60	770 gr.
27.	5,850,000	61	

Aqueous extract. The dried, brownish, crumbly residue left after treatment with alcohol and ether was ground in a mortar with 30 CC normal salt solution. Four injections were given as shewn in experiment 14. In each case an undoubted rise in corpuscles and haemoglobin took place.

Experiment 14. Male rabbit, 2 months old; rice diet. Steady fall in corpuscles and haemoglobin; gradual rise in weight. Four subcutaneous injections of aqueous extract of marrow, all giving positive results.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Mar. 21.	6,563,000	65	1110 gr.
22.	Rice diet begun.		
24.	6,583,000	65	1200
28.	6,400,000	65	1110
30.	6,312,000	63	1170
April 2.	5,875,000	62	1170
4.	5,800,000	61	1200
6.	6,000,000	61	1220
9.	5,800,000	61	1230
11.	5,362,500	55	1220
17.	5,487,000	59	1270
19.	5,300,000	58	1220
Subcutaneous injection 5 CC. aqueous extract.			
20.	6,637,500	65	1220
21.	5,900,000	62	1270
22.	5,337 000	60	1270

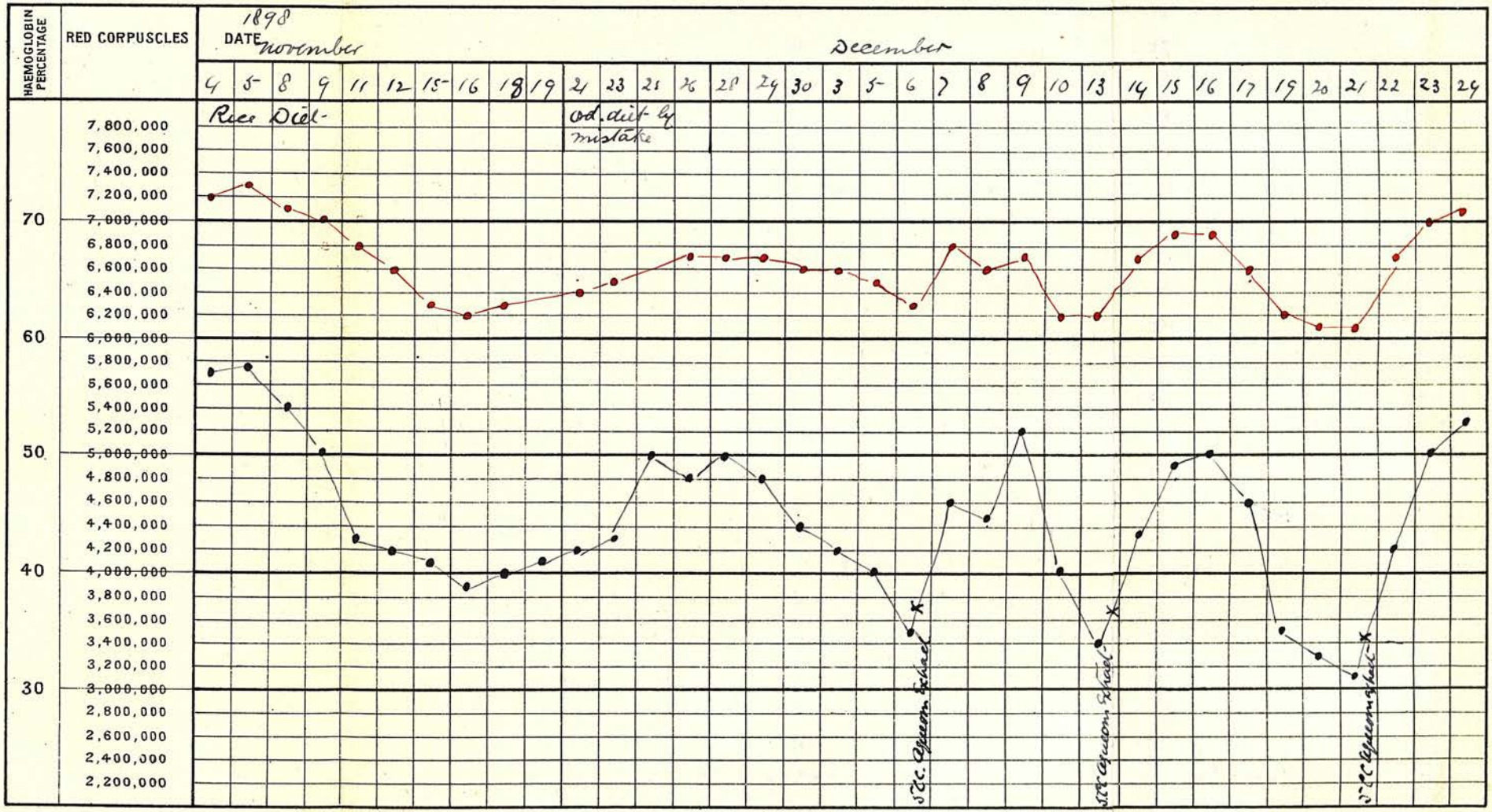
Experiment 14. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
April 22.	Subcutaneous injection 5 CC. aqueous ext.		
23.	5,900,000	62	1230
24.	5,000,000	60	1230
May 1.	4,250,000	53	1320
Subcutaneous injection 5 CC. aqueous extract.			
2.	4,650,000	60	1230
3.	5,200,000	61	1200
4.	4,000,000	48	1300
5.	3,337,000	48	1300
Subcutaneous injection 10 CC. aqueous extract.			
6.	3,650,000	52	1300
7.	5,000,000	60	1330
8.	4,537,000	53	1320
9.	4,812,000	55	1320
10.	4,450,000	50	1330
11.	4,650,000	50	1340
13.	3,000,000	50	1360
14.	Ordinary diet.		
15.	3,000,000	49	1370
17.	4,000,000	52	1400

At this point my supply of marrow failed, and for the material to make the remaining observations I am indebted to Messrs Burroughs, Wellcome, and Co. The marrow was treated as described above, and injections of aqueous extract were given to two animals: in both cases a distinct stimulating influence on the formation of red corpuscles was noted.

Experiment 15. Young male rabbit; rice diet. Steady fall in corpuscles and haemoglobin; weight almost stationary. Subcutaneous injections of aqueous extract with positive result.

Experiment 15. Anaemic rabbit.

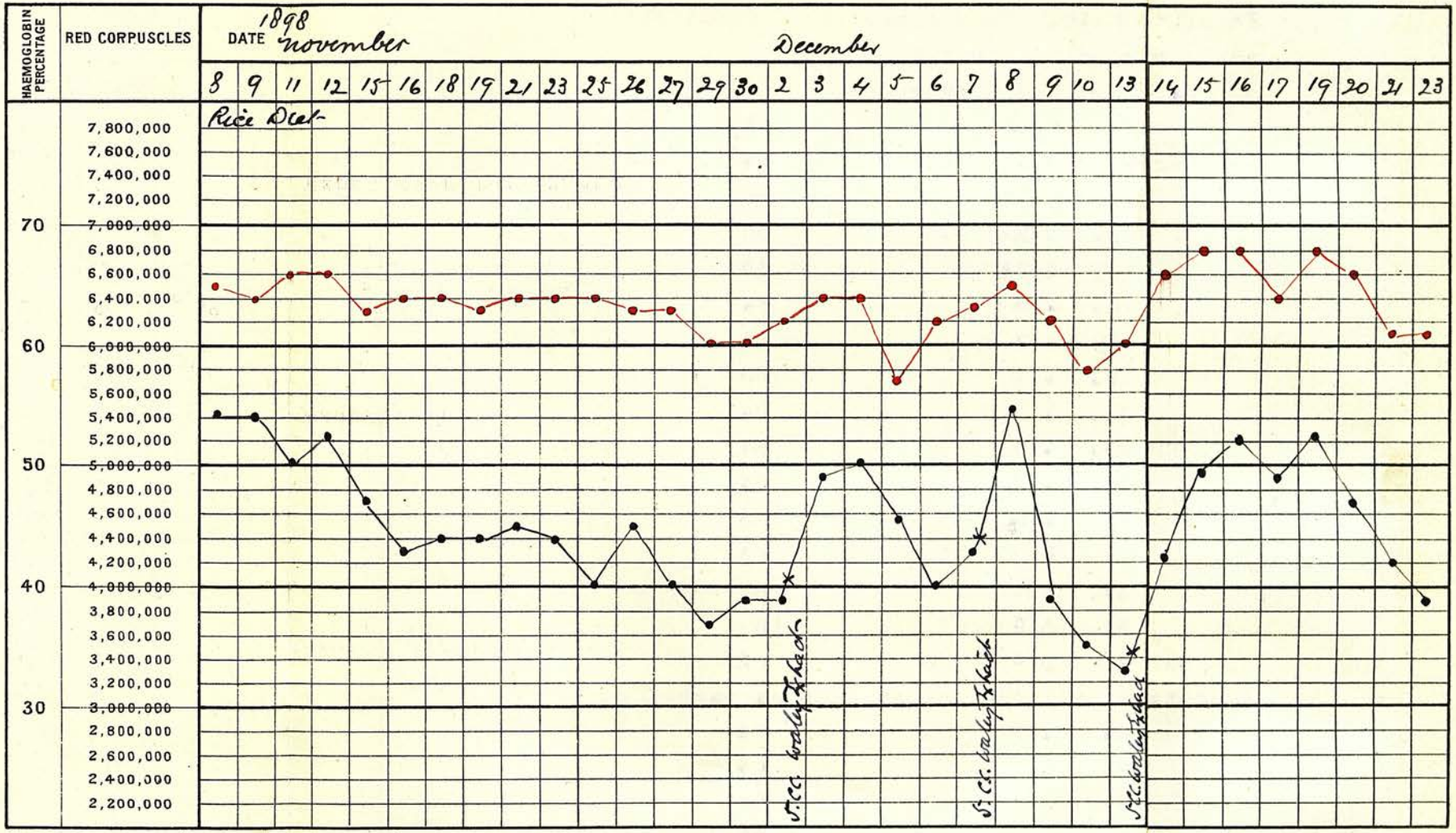


Corpuscles falling; downward course interrupted by an accidental return to ordinary diet. Three injections aqueous extract of marrow, each fol-lowed by rise in corpuscles and haemoglobin.

Experiment 15.(continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 4.	5,700,000	72	2050
5.	5,741,000	73	
8.	5,437,000	71	
9.	5,000,000	70	2080
11.	4,320,000	68	
12.	4,236,000	66	
15.	4,100,000	63	
16.	3,900,000	62	
18.	4,000,000	63	2000
19.	4,190,000	-	
21.	4,200,000	64	From this date until the 26th ordinary diet was accidentally given to the animal: hence the rise in the corpuscles.
23.	4,300,000	65	
25.	5,000,000	-	
26.	4,800,000	67	2050
28.	5,000,000	67	
29.	4,800,000	67	
30.	4,410,000	66	
Dec. 3.	4,200,000	66	
5.	3,900,000	65	2100
6.	3,500,000	63	Subcutaneous injection aqueous extract= 1·5 gr. marrow.
7.	4,600,000	68	
8.	4,430,000	66	
9.	5,250,000	67	
10.	4,000,000	62	2000
13.	3,400,000	62	Subcutaneous injection aqueous extract=1·5 gr. marrow.
14.	4,380,000	67	
15.	4,908,000	69	
16.	5,000,000	69	
17.	4,600,000	66	
19.	3,500,000	62	
20.	3,260,000	61	2050
21.	3,160,000	61	Subcutaneous injection aqueous extract=1·5 gr.marrow
22.	4,200,000	67	2050

Experiment 16. Anaemic rabbit.



Corpuscles falling. Three injections aqueous extract of marrow, each followed by rise in corpuscles and haemoglobin.

Experiment 15. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Dec. 23.	5,000,000	70	
24.	5,300,000	71	
26.	5,000,000	68	2000 gr.

Experiment 16. Young rabbit. Conditions and results as in preceding experiment.

Date.	Red corpuscles.	Haemoglobin	Weight.
Nov. 8.	5,437,000	65	2550 gr.
9.	5,400,000	64	
11.	5,020,000	66	
12.	5,270,000	66	
15.	4,710,000	63	2350
16.	4,300,000	64	
18.	4,300,000	64	
19.	4,400,000	63	
21.	4,500,000	64	
23.	4,400,000	64	
25.	4,010,000	64	2450
26.	4,500,000	63	
27.	4,000,000	63	
29.	3,700,000	60	2400
30.	3,900,000	60	
Dec. 2.	3,910,000	62	
Subcutaneous injection aqueous extract=1 gr. marrow.			
3.	4,900,000	64	2450
4.	5,000,000	64	
5.	3,580,000	57	
6.	4,000,000	62	2420
7.	4,300,000	63	
Subcutaneous injection aqueous extract=1 gr. marrow.			
8.	5,470,000	65	
9.	3,900,000	62	
10.	3,500,000	59	2410
13.	3,300,000	60	
Subcutaneous injection aqueous extract=1 gr. marrow.			
14.	4,220,000	67	2400

Experiment 16. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Dec. 15.	4,950,000	68	
16.	5,200,000	68	
17.	4,900,000	64	
19.	5,270,000	68	
20.	4,700,000	66	
21.	4,100,000	61	
23.	3,900,000	61	2400 gr.

V. Action of other substances on blood-formation.

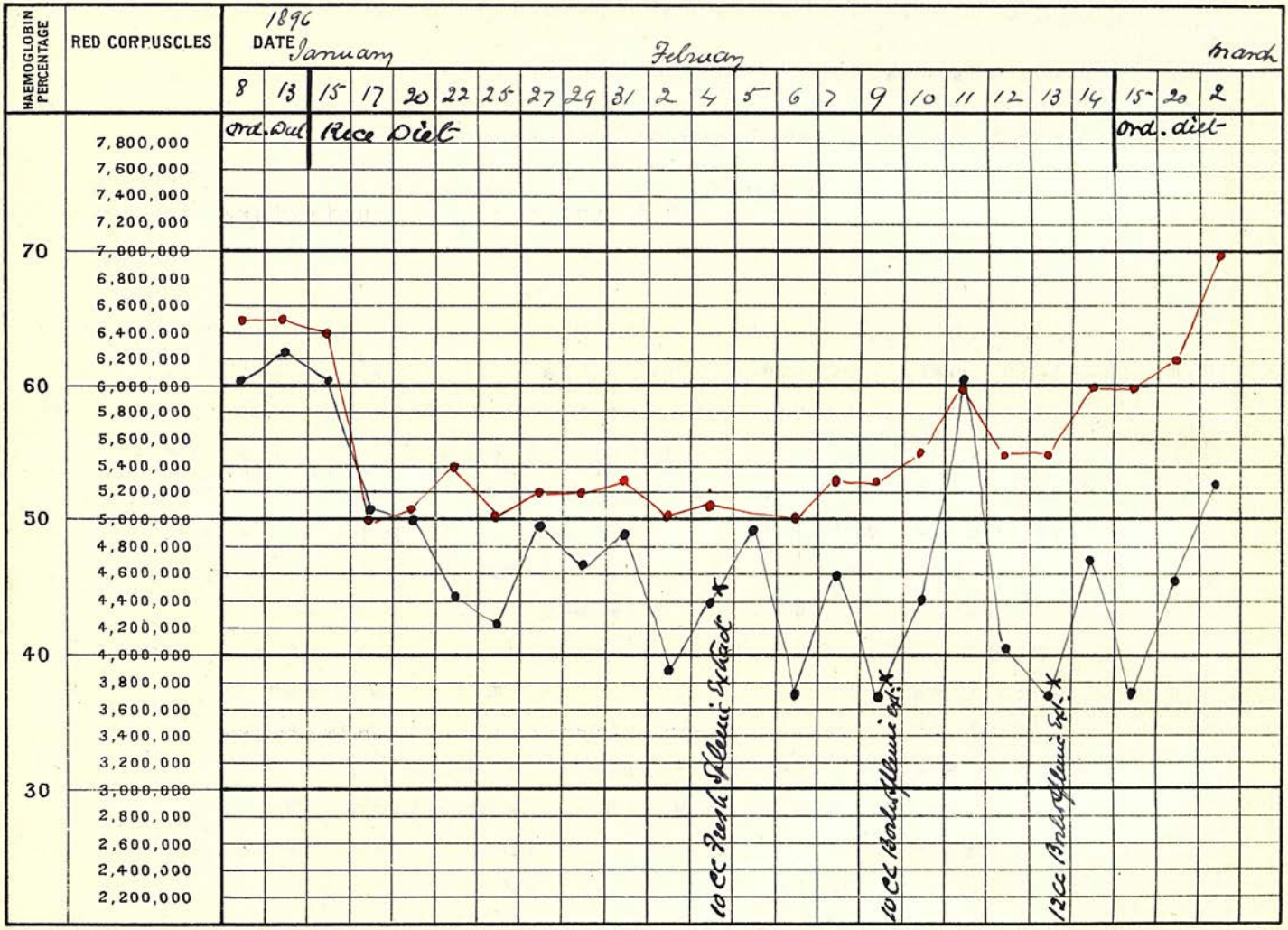
A few observations were made on the actions of splenic extract, blood-serum, and haemoglobin.

(1) Splenic Extract.

Of this there is little to be said. Danilewsky (op. cit.)obtained much the same results by injecting splenic extract as with bone-marrow — a marked rise in the number of corpuscles and amount of haemoglobin — and this in normal as well as anaemic animals, and with both fresh and boiled extract. In his original paper in 1895, Danilewsky states that further observations are being made with the view of isolating the active substance, and that the action of splenic extract on animals whose spleens have been removed is also the subject of investigation. So far as I can discover, however, no later communication on the matter has been made.

My two earliest experiments were made with splenic

Experiment 17. Anaemic Rabbit.



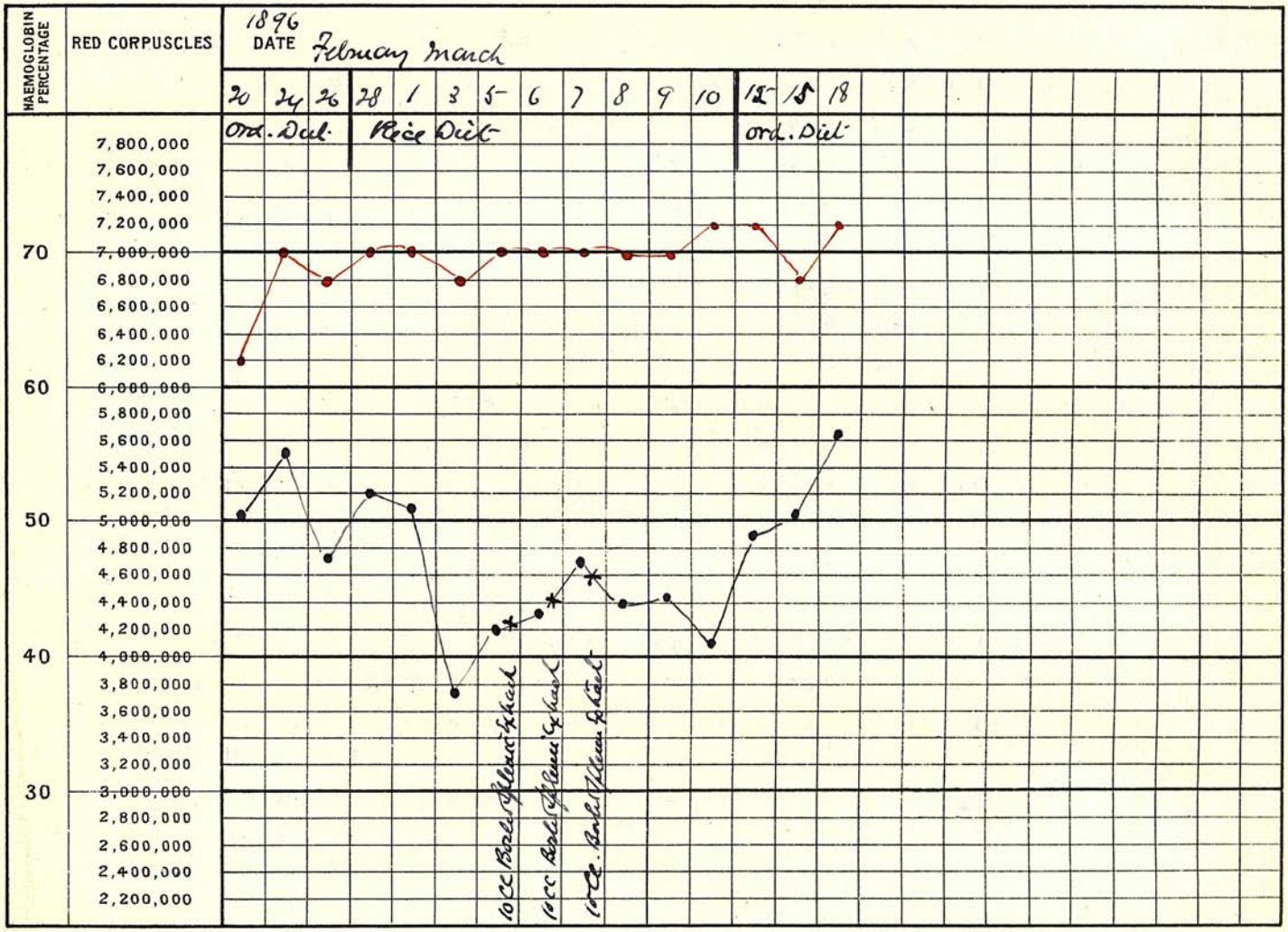
Corpuscles and haemoglobin falling irregularly. One injection fresh, and two of boiled, splenic extract. Result inconclusive.

extract prepared by grinding 100 grammes fresh spleen (obtained with antiseptic precautions)with 100 CC. saline solution. In neither case did I detect any evidence of a definite action on the corpuscles. The temporary rise seen in experiment 17 must, I think, be regarded as accidental and probably fallacious. The somewhat variable number of corpuscles recorded is, I believe, to be ascribed to the fact that I did not, at that time, fully appreciate the importance of obtaining the blood while the vessels were fully dilated.

Experiment 17. Adult male rabbit; rice diet from 15th inst. onwards. One intra-peritoneal injection of fresh, and two of boiled splenic extract. No positive result. The rise on February 10th is probably accidental; the animal had eaten nothing during the preceding twenty-four hours, and it is possible that some concentration of the blood may have taken place on that date. (Note. This was the only occasion, except in the fatal cases alluded to, on which an intra-peritoneal or other injection seemed in any way to affect the health of an animal. No tenderness or other sign of discomfort was ever produced)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Jan. 8.	6,030,000	65	2068 gr.
13.	6,280,000	65	
15.	6,052,000	64	
	Rice diet begun.		
17.	5,112,000	50	2080
20.	5,000,000	51	
22.	4,460,000	54	
25.	4,280,000	50	2040
27.	4,960,000	52	
29.	4,720,000	52	
31.	4,900,000	53	2060
Feb. 2.	3,920,000	50	

Experiment 18. Anaemic rabbit.



Corpuscles falling. Three injections boiled splenic extract.

Result negative.

Experiment 17. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Feb. 4.	4,460,000	51	2070 gr.
Intra-peritoneal injection 10 CC. fresh splenic extract.			
5.	4,920,000	-	
6.	3,720,000	50	
7.	4,640,000	53	2080
9.	3,720,000	53	
Intra-peritoneal injection 10 CC. boiled splenic ext.			
10.	4,430,000	55	
11.	6,140,000(!)	60	2020
12.	4,060,000	55	
13.	3,760,000	55	
Intra-peritoneal injection 12 CC. boiled splenic ext.			
14.	4,720,000	60	
15.	3,780,000	60	
16.	Ordinary diet resumed.		
20.	4,586,000	62	2160
Mar. 2.	5,292,000	70	
6.	5,560,000	-	

Experiment 18. Adult male rabbit; rice diet from 28th inst. onwards. Fall in red corpuscles. Three intra-peritoneal injections boiled splenic extract on successive days. Result negative.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Feb. 20.	5,040,000	62	1645 gr.
24.	5,560,000	70	
26.	4,780,000	68	
28.	5,212,000	70	1750
Mar. 1.	5,160,000	Rice diet begun. -	
3.	3,760,000	68	1750
5.	4,220,000	70	
Intra-peritoneal injection 10 CC. boiled splenic ext.			
6.	4,360,000	70	
Intra-peritoneal injection 10 CC. boiled splenic ext.			
7.	4,720,000	70	1680
Intra-peritoneal injection 10 CC. boiled splenic ext.			

Experiment 18. (continued)

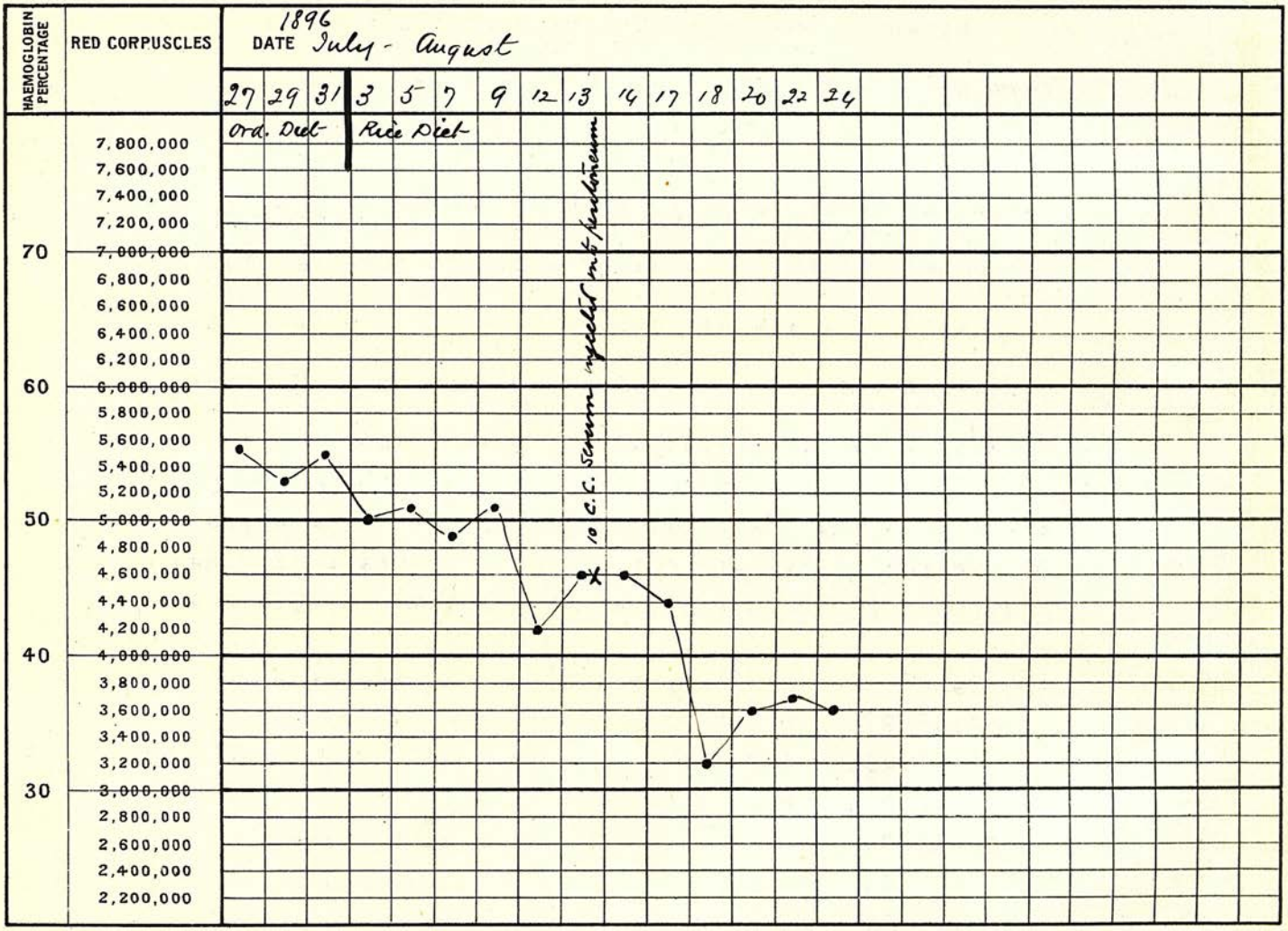
Date.	Red corpuscles.	Haemoglobin.	Weight.
Mar. 8.	4,400,000	70	1700 gr.
9.	4,450,000	70	
10.	4,150,000	72	
12.	4,900,000	72	
15.	5,080,000	68	
18.	5,640,000	72	1756

(2) Blood Serum.

It is now an accepted fact, as was shewn by Landois many years ago, that the blood serum of animals (in particular of dogs), when injected into the veins of an animal of a different species, breaks down and destroys the blood corpuscles. Maragliano (23) has proved that, in disease, human serum causes what he calls "necro-biotic changes" in human red corpuscles. He studied the action of the serum of all forms of essential anaemia, of carcinoma, of plumbism, of malaria, of nephritis, and of many other diseases, and found that it first produced poikilocytosis and then broke down the cells, not only of a healthy person, but also of the patient from whom it was obtained.

Bearing these facts in view, it occurred to me that if one injected the serum of a healthy animal into the veins of one which was anaemic, and whose corpuscles shewed poikilocytosis, in consequence of its having been deprived for some time of nitrogenous food, it was quite likely that some action in the way

Experiment 19. Anaemic rabbit.



Corpuscles falling steadily. Intraperitoneal injection rabbit-serum. Marked diminution in corpuscles.

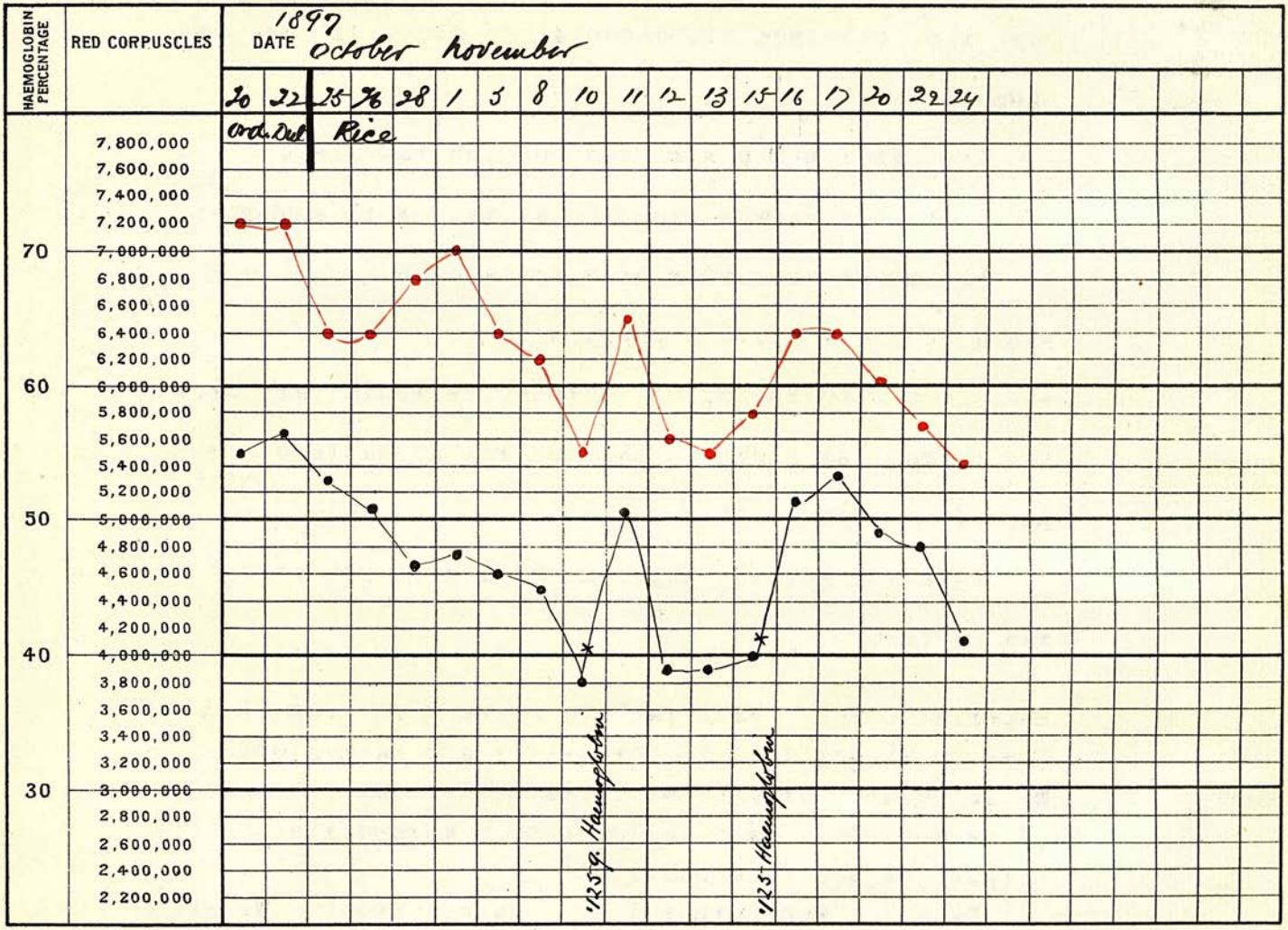
of increasing the red corpuscles might take place. And the expectation of this was in a manner strengthened by the undoubted benefit accruing in certain cases of pernicious anaemia(as recorded by Brackenridge, Affleck, and others)from the transfusion of human blood.

A rabbit was therefore bled to death under chloroform and the blood collected in a sterile vessel. This was placed in the ice-chest, and in 48 hours the serum was decanted off for use. As my license did not permit of the necessary operation for transfusion into a vein, I was obliged to be content with injecting the serum into the peritoneal cavity. Contrary to what I had anticipated, the result was negative.

Experiment 19. Male rabbit; rice diet from August 3d onwards. Fall in corpuscles. Intra-peritoneal injection 10 CC. normal rabbit's serum. Result negative.

Date	Red corpuscles.	Haemoglobin.
July 27.	5,540,000	-
29.	5,300,000	-
31.	5,500,000	-
Aug. 3.	5,000,000	Rice diet begun.
5.	5,100,000	-
7.	4,900,000	-
9.	5,100,000	-
12.	4,200,000	-
13.	4,600,000	10 CC. serum injected.
14.	4,600,000	-
17.	4,400,000	-
18.	3,300,000	-
20.	3,605,000	-
22.	3,710,000	-

Experiment 20. Anaemic rabbit.



Corpuscles and haemoglobin falling. Two injections of haemoglobin solution, each followed by rise in red corpuscles and haemoglobin.

(3) Haemoglobin.

Three experiments on the action of haemoglobin (obtained from dog's blood) were made. It was dissolved in a 5 per cent solution of sodium carbonate in normal salt solution. Every 5 CC. of the deep port-wine coloured fluid contained about 125 mg. dry haemoglobin.

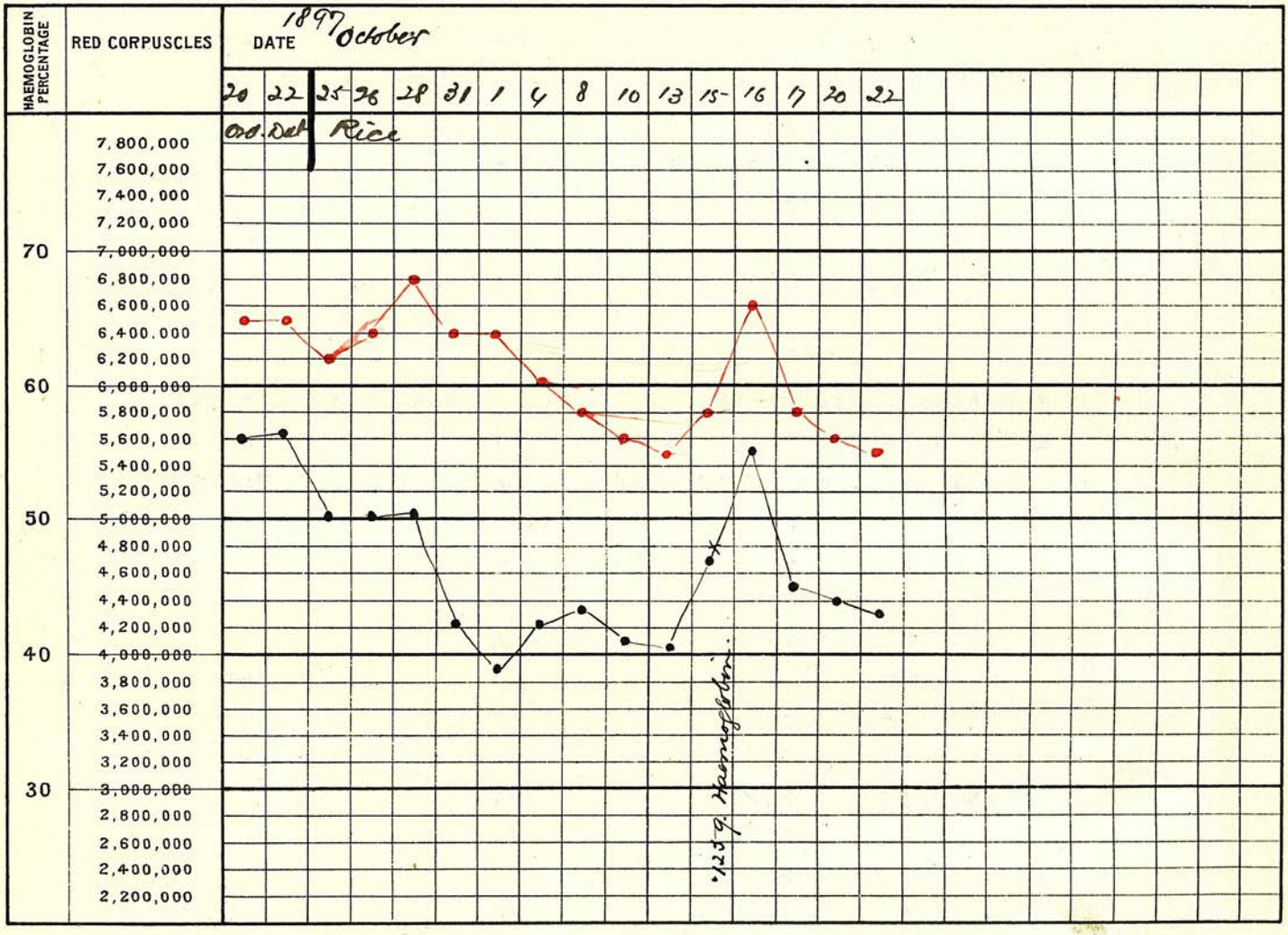
The experiments were made on two rabbits and one dog. In the former case the animals were anaemic; in the latter, a number of subcutaneous injections was given to a healthy puppy with the view of seeing whether the course of the corpuscles would vary from the normal, as shewn in the control animal(another pup of the same litter.

In all the three experiments a distinct effect was noticed.

Experiment 20. Male rabbit; rice diet from 22nd inst. Steady fall in corpuscles and haemoglobin. Weight stationary. Two intra-peritoneal injections of haemoglobin, each followed by a marked rise in the corpuscles and haemoglobin.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Oct. 20.	5,500,000	72	1600 gr.
22.	5,612,000	72	
	Rice diet begun.		
25.	5,387,000	64	
26.	5,050,000	64	
28.	4,638,000	68	1550
Nov. 1.	4,762,000	70	
5.	4,600,000	64	
8.	4,500,000	62	1630

Experiment 20. Anaemic rabbit.



Corpuscles and haemoglobin falling. Injection of haemoglobin, followed by rise in corpuscles and haemoglobin.

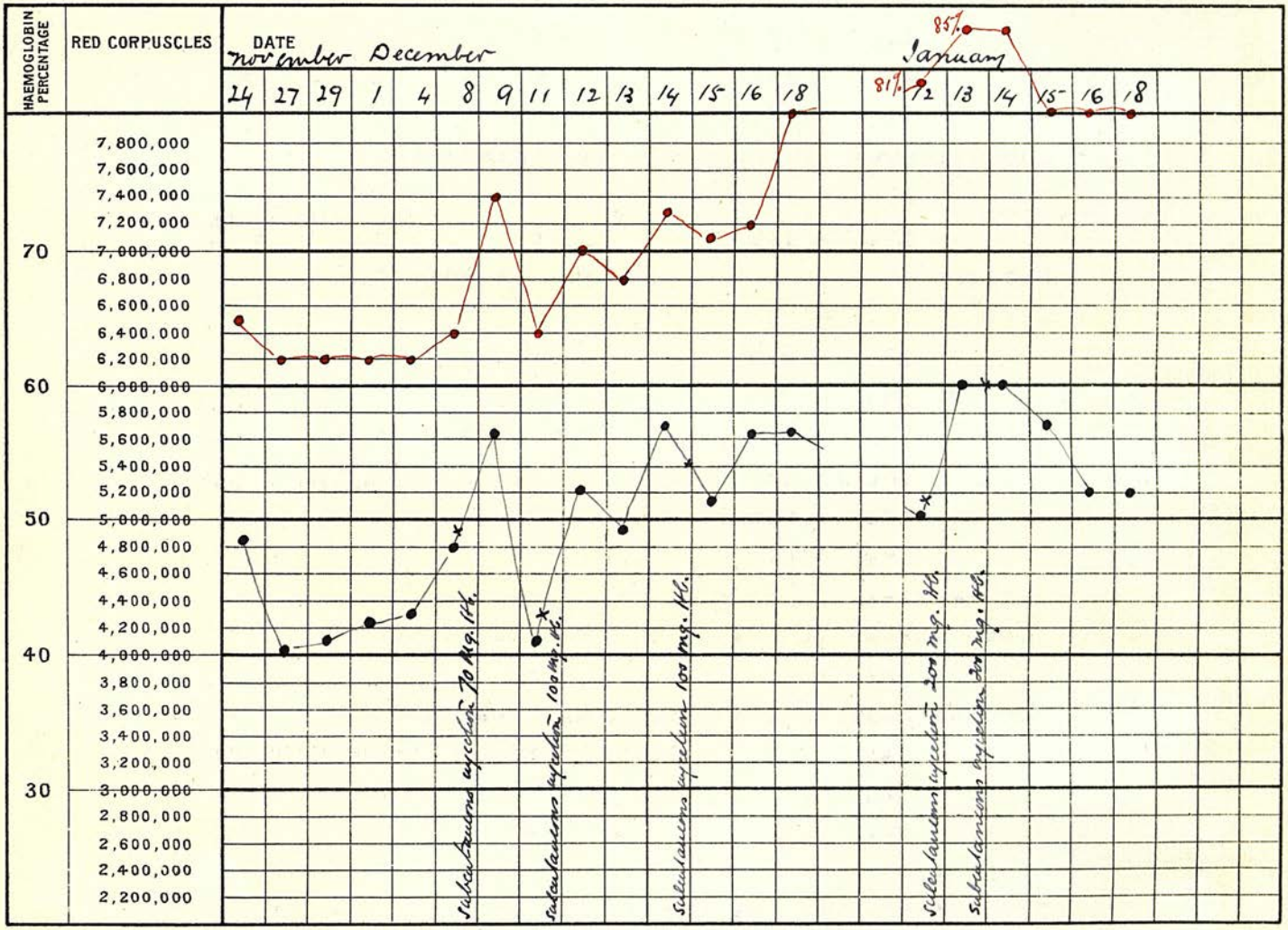
Experiment 20. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 8.	4,500,000	62	1630 gr.
10.	3,800,000	55	
Subcutaneous injection 5 CC. haemoglobin solution.			
11.	5,025,000	65	
12.	3,900,000	56	1550
13.	3,900,000	55	
15.	4,000,000	58	1480
Subcutaneous injection 5 CC. haemoglobin solution.			
16.	5,140,000	64	
17.	5,183,000	64	
20.	4,900,000	60	
22.	4,800,000	57	1650
23.	4,200,000	54	

Experiment 21. Rabbit; conditions and results same as in preceding observation.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Oct. 20.	5,600,000	65	1500 gr.
22.	5,625,000	65	
	Rice diet begun.		
25.	5,000,000	62	
26.	5,000,000	64	1450
28.	5,110,000	68	
31.	4,210,000	64	1520
Nov. 1.	3,912,000	64	
4.	4,210,000	60	
8.	4,350,000	58	
10.	4,162,000	56	
13.	4,037,000	55	1490
15.	4,700,000	58	
Subcutaneous injection 5 CC. haemoglobin solution.			
16.	5,525,000	66	
17.	4,500,000	58	
20.	4,400,000	56	
22.	4,275,000	55	1550

Experiment 22. Normal puppy.



Five subcutaneous injections haemoglobin. Note general rise in corpuscles and especially in haemoglobin, and that the effect was present on Jan. 12th, when more than three weeks had elapsed since last injection. Compare with experiment 23.

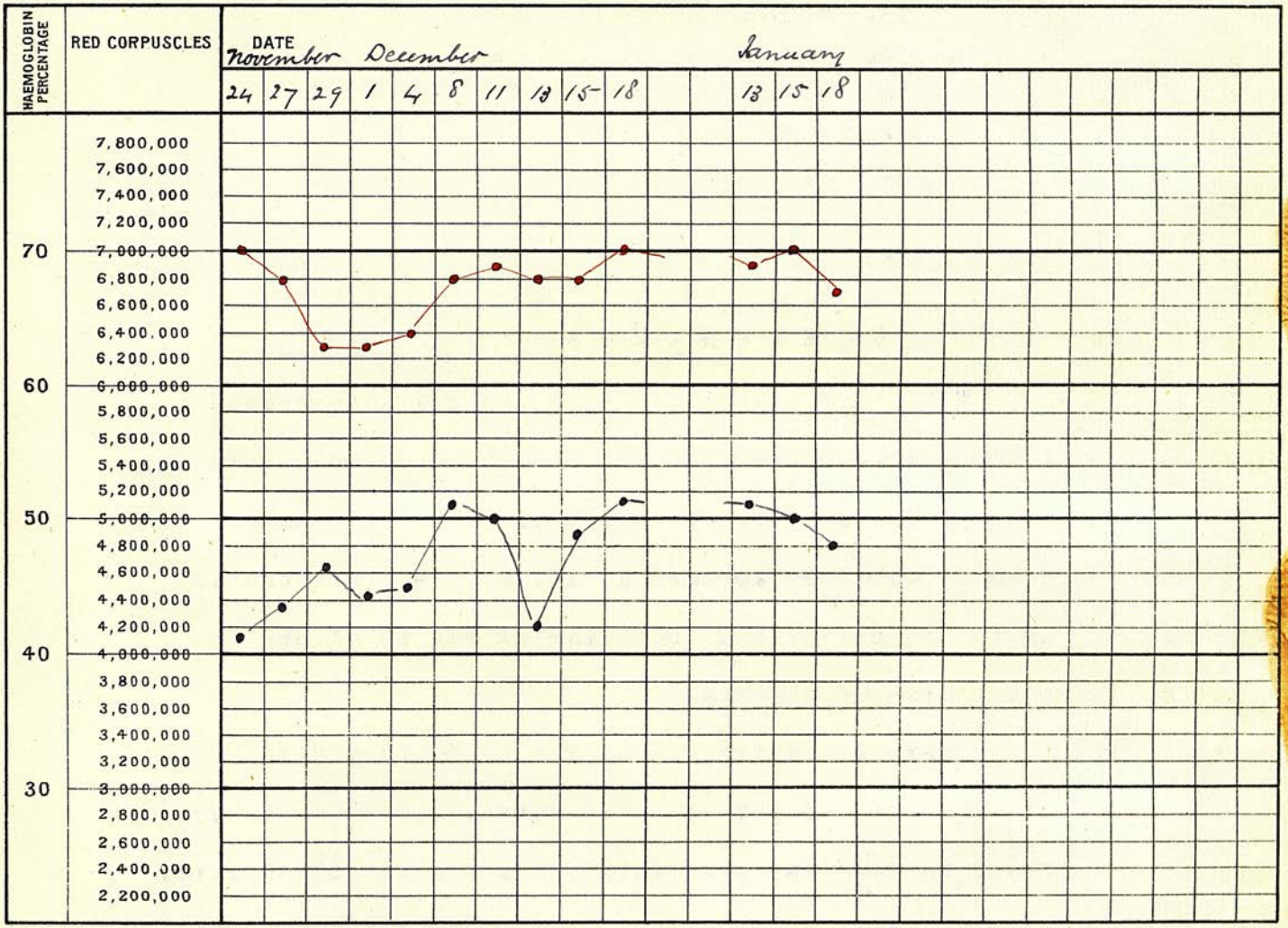
Experiment 22. Male fox-terrier pup; ordinary diet. Five subcutaneous injections of haemoglobin. By comparing the course of the corpuscles and haemoglobin with that in the control (Experiment 23.), it will be seen that here there is a considerable rise in the former, and a very marked increase in the latter.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 24.	4,850,000	65	3000 gr.
27.	4,000,000	62	
29.	4,075,000	62	3470
Dec. 1.	4,120,000	62	
4.	4,325,000	62	3950
8.	4,800,000	64	
Subcutaneous injection 70 mg. haemoglobin.			
9.	5,612,000	74	
11.	4,112,000	64	4700
Subcutaneous injection 100 mg. haemoglobin.			
12.	5,210,000	70	
13.	4,925,000	68	
14.	5,725,000	73	
Subcutaneous injection 100 mg. haemoglobin.			
15.	5,187,000	71	
16.	5,626,000	72	
18.	5,660,000	70	
Jan. 12.	5,000,000	81	
Subcutaneous injection 200 mg. haemoglobin.			
13.	6,000,000	85	
Subcutaneous injection 200 mg. heamoglobin.			
14.	6,000,000	85	
15.	5,700,000	80	
18.	5,200,000	80	

Experiment 23. Male fox-terrier pup. Control to preceding.

Date.	Red corpuscles.	Haemoglobin.	Weight.
Nov. 24.	4,150,000	70	3800 gr.
27.	4,375,000	68	
29.	4,650,000	63	
Dec. 1.	4,425,000	63	

Experiment 23. Normal puppy. Control to experiment 22.



Experiment 23. (continued)

Date.	Red corpuscles.	Haemoglobin.	Weight.
Dec. 1.	4,425,000	63	4570 gr.
4.	4,500,000	64	
8.	5,137,000	68	
11.	5,000,000	69	4900
13.	4,200,000	68	
15.	4,900,000	68	
18.	5,162,000	70	
Jan. 13.	5,100,000	69	
15.	5,000,000	70	
18.	4,900,000	67	

C O M M E N T A R Y .

With these experiments my observations cease. I am conscious that the results ahve not been very striking, and that further investigation of the matter is needed, but they appear, at all events, to encourage us in the belief that bone-marrow may be of use in some forms of anaemia.

There are a few points yet to be considered. It will be noticed that the two substances which I have found to exercise a stimulating action on blood-formation — or at least on the number of corpuscles present — are haemoglobin and extract of bone marrow.

To take the latter of these first:- It will be observed that haemoglobin appears to exert a marked influence on the blood of a healthy growing animal, and to raise the level of the corpuscles, and, even

more markedly, of the haemoglobin, far above that in the control animal. The effect, moreover, is one of some duration; for (Exp. 22.), at the end of the period from the 14th December to the 12th January when no injections were being given, the corpuscles were found to be nearly a million, and the haemoglobin almost twenty per cent, above the former level in the animal inquestion, as well as in the control.

My observations with haemoglobin are not sufficiently numerous to allow me to speak with any certainty of its action, but if experiments 20 and 21 be compared with experiment 22, it will be seen that the relative rise in the haemoglobin in the two former (i.e. in the case of the anaemic rabbits) is not so great as that in the dog. In fact, the action as shewn in the charts is very similar to that of marrow extract. Otherwise, however, the difference between the action of marrow and that of haemoglobin is striking: the former (save in the somewhat inconclusive experiment 8) seems to have little effect on the blood of healthy animals, although the action is distinct if they are anaemic; the latter, on the other hand, has a very decided action in the blood in health, chiefly in the direction of increasing the haemoglobin. It is almost impossible, I think, to escape the conclusion that the haemoglobin is absorbed and utilized as such, and it

seems possible that further investigation along the lines of experiment 23. would help to solve some of the vexed questions surrounding the absorption of iron into the body.

It may be asked, If haemoglobin have such an action on the blood, why should a fresh animal extract — that of the spleen for example — which certainly must contain haemoglobin, not exert a similar influence? Two considerations seem to offer an explanation. In the first place, the amount of haemoglobin in the injections of splenic extract used cannot possibly have been as much as 125 - 200 mg. of the dried substance; and, secondly, it is not impossible that the spleen may contain some haemolytic property. I have, however, no data to go upon to enable me to come to any conclusion in this respect, my experiments having been chiefly directed towards the action of bone marrow.

The only further comment to be made on the negative action of splenic extract and blood serum is, that we have here a proof that the rise in corpuscles produced by the marrow was not simply the result of the introduction of nitrogenous matters into the body of an animal otherwise deprived of them. The two substances may, indeed, be regarded as in some sense controls to the action of bone marrow.

Bone marrow :-

When an increase in the red corpuscles, especially such a sudden and decided increase as some of the experiments shew, is observed, it may obviously be due (1) to an absolute gain in the red corpuscles, (2) to a redistribution of those already present, so that the peripheral circulation contains more, and the deep circulation fewer, or (3) to a concentration of the blood by the withdrawal of some of its fluid constituents.

In the case of the white corpuscles, some forms of leucocytosis have been explained on the second of these grounds, but, so far as I know, the red corpuscles have never been shewn to behave in an analogous manner. As it seemed unlikely that one organic substance should act in such a fashion — by causing an abnormal distribution of the erythrocytes — while another and very similar one did not do so, and as all estimations of red corpuscles have reference solely to those circulating in the skin capillaries, I did not attempt to investigate this matter. The most likely fallacy was that the injections might have caused diarrhoea, and thereby concentrated the blood. This was never observed, nor was there any ground (save as noted in experiment 17.) for imagining that a withdrawal of fluid from the body occurred. I think, therefore, that we are justified in believing that an actual increase in the

formed elements took place.

With the progress of the anaemia produced by the rice diet, the red cells became deformed, and eventually a fairly well-marked poikilocytosis was present. There was a certain amount of languor, and the animals were inclined to mope in the corner of the cage. Now, although the marrow increased the number of the corpuscles, their form was not correspondingly improved, nor was there any betterment of the general condition of the animal. A return to the ordinary diet, however, brought them back to the normal condition in the course of a week or a fortnight. The feature of the action of the bone-marrow was the sudden and very large increase in the number of the red corpuscles, and it occasionally seemed, notably in experiment 14., as if the subsequent fall was more rapid than would otherwise have been the case.

No such rises in the corpuscles have ever been recorded clinically as results of treatment with marrow. But, putting aside the differences in the form of the anaemia in the rabbit and in man, it must be noted that the doses of marrow used were relatively enormous. The effect was, it seemed to me, that of a sudden and powerful stimulus to the haemopoietic function.

As to the active principle, I am able to say little. It is right (though perhaps scarcely necessary) to point

out that the saline solution used in my experiments has no effect on increasing the red corpuscles. It is true that Maurel(27)states that subcutaneous injections of 30 CC. per kilo of body weight of •7 per cent salt solution favour "the reconstitution of the blood". Smaller doses, however, have no such action, and I find that the few centigrammes injected in my experiments are quite negligeable.

The chemistry of bone-marrow appears to have been worked at only by Forrest(24). His results are as follows:- "(1) Proteose(albumose)and peptone are absent . . . . (2) Albumin is obtainable in the merest traces, and is coagulated by a temperature of 70-75 deg. (3) The two proteids which are obtainable in a saline extract are one which is coagulated at 47 - 50 deg., and another at 60 -65 deg. . . . . (4) The former of these two proteids is a globulin; it contains practically no phosphorus in its molecule. (5) The second . . . contains phosphorus. It gives all the tests characteristic of a nucleo-albumin . . . . (7) It resembles other nucleo-albumins in producing intra-vascular clotting . . . . in rabbits . . . . (10) . . . . the proteids of red marrow are similar to those of other richly cellular structures."

Danilewsky(op. cit.)expresses the view that the active substance, according to his observations, may

turn out to be lecithin. His reason for this appears to be based on his work on the stimulative action of that substance on the frog embryo, and also on his observation that lecithin, when injected subcutaneously into dogs, increases the corpuscles from 800,000 to 1,000,000 above normal(26). Lecithin, however, cannot be the active agent here, since it is absent the aqueous extract used in experiments 14., 15., and 16. At an early stage of the work it was suggested to me that nucleic acid might be the active principle; I therefore tried the effect of one or two injections of this material, but without any result so far as the red corpuscles and haemoglobin were concerned. The figures were entirely negative, and for this reason I do not give the experiments in detail.

The aqueous extract got after extraction with alcohol was examined for the presence of organic and inorganic iron by Macallum's method(27), and was found to be entirely free from it. Dr Noel Paton was kind enough to examine the same extract for proteids, and found that it contained a deutero-proteose in small amount(thus contradicting Forrest's observation), but no other proteid. Whether this deutero-proteose is the active principle, I am not in a position to say.

P A R T I I.

On the Splenic Anaemias of Infancy.

During the past eight or ten years a great deal has been written on the anaemias of infancy. These are for the most part secondary, well-marked chlorosis, pernicious anaemia, and leucocythaemia being rare, and not, in general, deviating to any extent from what we are accustomed to meet with in adult life. Secondary anaemia, however, is extremely common, and may be said to differ from the adult type (1) in the frequency with which nucleated red corpuscles appear in the blood; (2) in the ease with which leucocytosis develops; and (3) in the frequency with which it is accompanied by enlargement of the spleen. It is obvious, therefore, that the appearance of the blood may differ very considerably in different cases.

I have had an opportunity of examining the blood of a considerable number of infants suffering from splenic anaemia, or, more correctly perhaps, anaemia with splenic enlargement, and have elsewhere discussed the general clinical features of the condition(28). Here, I wish to describe and illustrate the blood changes more fully than I have hitherto done. Some of the cases were seen in hospital; these I was able to watch and to examine on more than one occasion; others(the majority)were seen as out-patients or dispensary patients, and often passed from under observation. In many of these, circumstances precluded the enumeration of the corpuscles -- all that could be done was to prepare and stain films of the blood. It seems to me,

however, that the examination of films is really more important than enumeration; it has the advantage, too, of being easy to carry out whento count the corpuscles would be difficult or impossible.

In what follows I have selected what seem to me average cases illustrating the various degrees of blood change which may be met with. After having having described these, I shall go on to speak of the nature of splenic anaemia in infancy.

The Blood in Infancy.

Of late years a great deal of work has been done on this subject, and, though differences of opinion may exist on some of the minor details, the general features of the blood of infants and young children are now sufficiently well known. The most important recent papers on the subject are those of Alt and Weiss (29), Fischl(30), Loos(31), Luzet(32), Silbermann(33), and Weiss(34). From a study of the literature, and from my own observations, the general facts seem to be somewhat as follows:-

1. Erythrocytes and Haemoglobin. As is well known, the red corpuscles are most numerous at, and immediately after, birth — 5,500,000 to 6,000,000 per cmm. being perhaps an average number — and they rise slightly during the first two days of life. The number then descends gradually until about the second year, and thereafter slowly rises to the adult figure. The cells

vary somewhat in size and shape, but apparently not to the degree stated by early writers. The principal point under discussion, however, is as to the occurrence of erythroblasts in the healthy infant. It would appear that this depende mainly on the stage of the development of the blood at birth — a stage which may or may not correspond exactly with the degree of maturity of the infant. In most healthy newly-born infants it seems certain that a few nucleated red cells are still present — say, from 2 - 4 to every hundred leucocytes. (Hutchison and Elder(35)). They are almost always normoblasts.

The percentage of haemoglobin is high at birth — 105 by Gowers' Haemoglobinometer — and steadily diminishes until the third week or so. It begins to rise again by the sixth month. From 75 to 80 per cent would appear to be a normal amount in infants and young children.

2. Leucocytes. According to v. Limbeck(36) and Rieder(37) the usual number of leucocytes in the fasting adult is about 8000 per cmm. In children between the ages of nine and fifteen it is about 9600(Rieder op. cit. p.19.) and in the newly-born child from 15,000 to 20,000. After birth there is a steady fall, which may go on until only 8000 or 9000 are present(Rieder, op. cit. p.78.), but as the child is fed the number again rises, and speedily reaches 10,000 to 12,000, a

Table I.

Percentages of Leucocytes at :-

	Birth	One Year	Adult
Mononuclear (small (lymphocytes))	27-30	50 - 55	24 - 30
Mononuclear (large)	10-30		3 - 6
Transitional	23	7	? 3
Polymorphonuclear	18 – 35	35 – 40	60
Eosinophile	·5 – 10	·5 - 7	·5 - 4

figure which may, I think, be regarded as normal during the first eighteen months or two years of life.

The various forms of leucocytes found require a moment's consideration. In the adult, we may take it that about 30 per cent are mononuclear, and about 70 per cent polymorphonuclear. But in the child the ratio is reversed, the polymorphonuclear being about 30 per cent, and the mononuclear about 70 per cent (Fischl, op. cit.). In the adult the majority of the mononuclear cells are the so-called "lymphocytes", the large mononuclear and transitional (uebergangs-) forms being much less numerous. In young children, however, the state of matters is different; the large mononuclear and transitional cells make up a much larger proportion of the total leucocytes present. The accompanying table (Table I.), compiled from various sources and checked by my own observations, will serve to indicate generally the numbers of the different forms of leucocytes present at different epochs of life. The figures are, of course, only approximate; it is, especially in children, often difficult to decide to class a given cell as a large or small mononuclear, or as a transitional or polymorphonuclear form. The eosinophile cells appear to be relatively abundant in childhood, but, beyond this fact, the relations of the granulations are not well known. Mononuclear leucocytes having a fine basophile granulation are, I think, fairly common — more common

certainly, than in the adult.

To sum up:- At birth the red corpuscles and haemoglobin are high, but gradually fall below the adult number, slowly rising after the second year; one or two normoblasts may be present during the first few days of life. The leucocytes are more abundant in the early years than later; at first the mononuclear forms predominate, and the large mononuclear and transitional types form a relatively large percentage of these.

Splenic Anaemia of Infancy.

It is perhaps advisable at this point to explain the general purport of my observations.

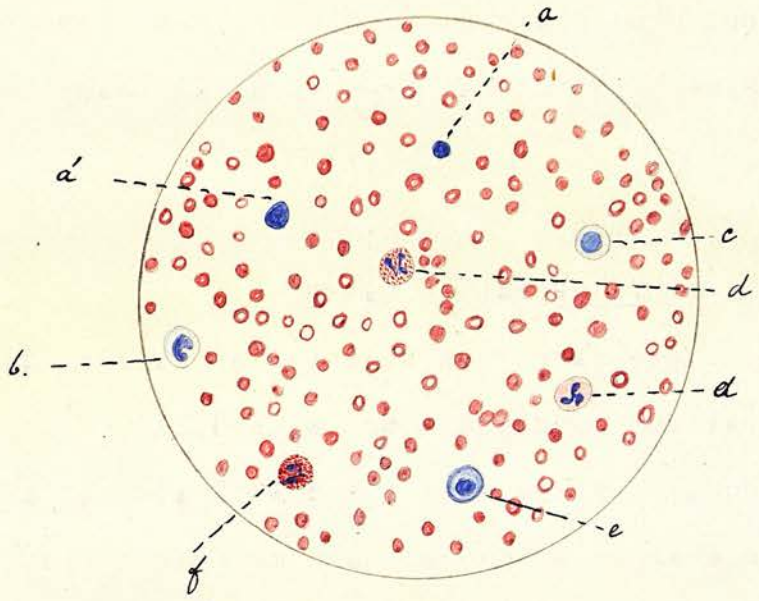
Attention was drawn to the subject of splenic anaemia in children by v. Jaksch in the year 1888, by the description of a condition to which he gave the name of Anaemia infantum pseudoleucaemica. In his most recent writing on the subject(37), the features said to be characteristic of the malady are, briefly stated, as follows:- Great enlargement of the spleen and possibly also of the liver, anaemia, and polymorphous leucocytosis — the white cells amounting at times to 60,000 or 80,000 per cmm. The disease is usually seen between the sixth month and the end of the second year. Luzet (op. cit.)added considerably to our knowledge of the condition. The characteristic feature of the blood (in addition to those already mentioned)is, according

to him, the presence of very numerous erythroblasts, many of them undergoing mitosis. Pathologically, he regards the disease as a reversion to the foetal type of haemopoiesis, and he describes the occurrence in the liver of haemopoietic cells exactly similar to those found in the foetus. Luzet's observations as to the condition of the liver have not been generally confirmed, but the appearances of the blood which he depicts have been corroborated by other writers, notably by Weiss(op. cit.).

My own view is that there is no justification for believing that this is a specific blood disorder. I find that if we examine a series of cases, clinically (i.e. apart from the microscopic change in the blood) resembling what is described by v. Jacksch as pseudo-leucaemic anaemia, all stages of blood change may be observed. We may find anything, from what is a practically normal condition, to a well-marked example of the blood change described above. The intermediate steps are simple anaemia with splenic enlargement, and anaemia with slight leucocytosis and splenic enlargement. And further, as recovery takes place, a case may pass through these different stages. Since I first began to examine cases in 1896, I find that many of the writers — Weiss, v. Limbeck, Cabot(38), etc. — have also come to the conclusion that the malady described by v. Jaksch is not a disease sui generis at all.

Figure I. case I.

Combined Field. Eosin-methylene blue ×400



a. a', small and large lymphocytes

b. "transitional" nucleus, with hyaline body

c. hyaline mononuclear

d. neutrophil polymorphonuclear leucocytes

e basophil (fuchsin) mononuclear

f. Eosinophil polymorphonuclear

As will be seen, however, the relation of the anaemia, the splenic enlargement, and the most common antecedent disease — rickets, are not at all easy to understand.

Illustrative Cases

(These cases have been selected as fairly illustrating the various conditions met with.)

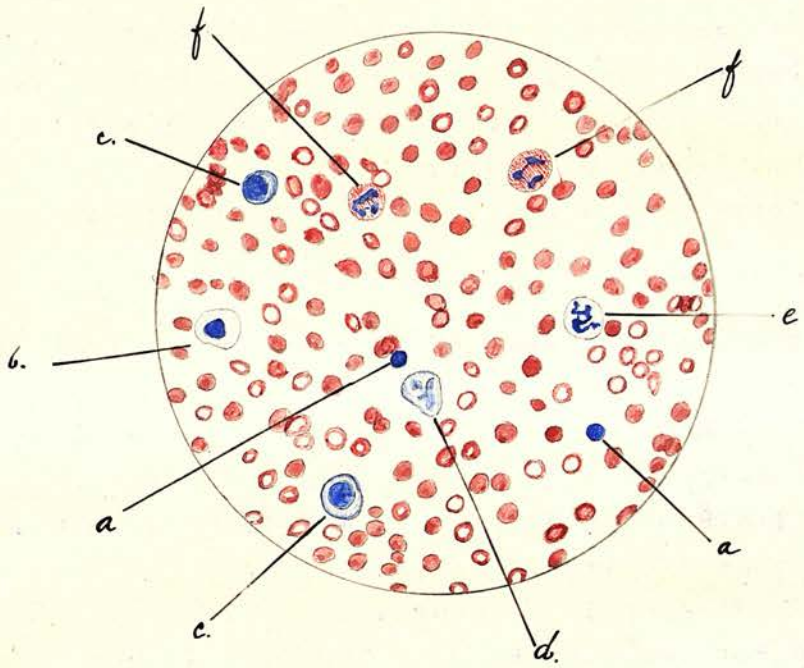
Methods. Films were fixed with alcohol and ether, and stained with eosin and methylene blue. In some cases the Ehrlich-Biondi triple stain was employed, but, as is a not uncommon experience, I had some in getting uniform results with it. In estimating the white cells care was taken to avoid a period during which a digestive leucocytosis might be expected. The number of erythroblasts is stated as that found in 100 successive fields of an oil-immersion lens. The figure can only be regarded as approximate.

Case I. J.J., aet. 18 months. Rachitis; slight pallor; spleen reaches iliac crest, liver is slightly enlarged.
Blood. 15/1/97. R-C. 4,900,000. W-C. 10,500. Hb. 80.
Anti-rachitic treatment.
15/4/97. Spleen just palpable, rickets better.
10/6/97. Spleen normal, no sign of anaemia.

Proportions of various cells.	15/1/97
Erythroblasts per 100 fields	-
Mononuclear leucocytes	55 per cent.
Transitional "	7 per cent.
Polymorphonuclear "	37 per cent.
Eosinophile "	1 per cent.

Figure II. Case II.

Combined Field. Eosm: Methylene blue. X 400.



a. lymphocytes
b. hyaline mononuclear
c. fine basophil mononuclear
d. transional basophile
e hyaline polymorphonuclear
f. polymorphonuclear with admixed fine acidophile (neutrophil) granules.

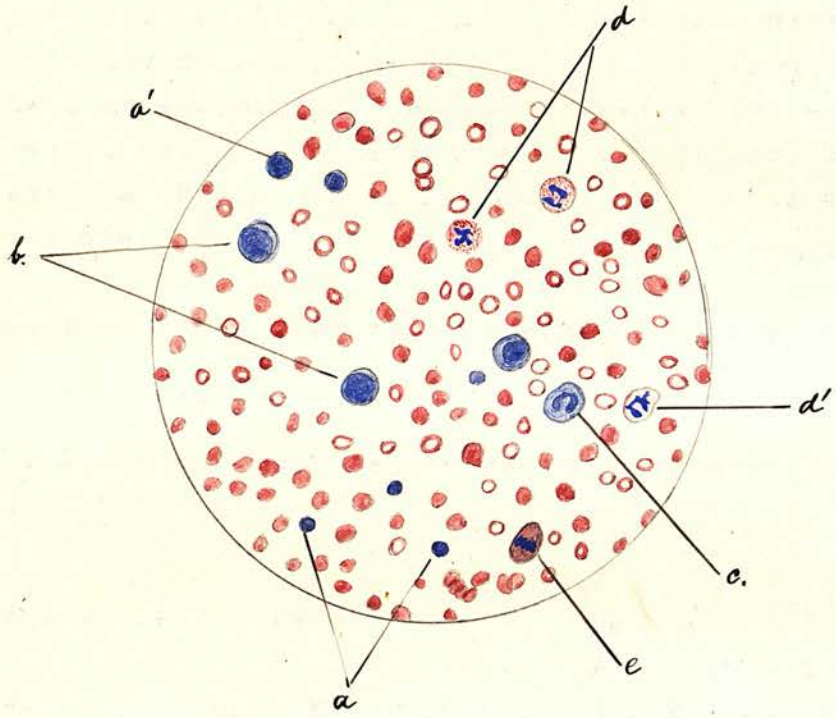
Microscopic Appearances.(Fig. I.) The cells are practically normal. We have the following forms of leucocytes:- (1) Lymphocytes of various sizes. (2) Mononuclear cells whose cell-body is hyaline or contains fine basophile granules. (3) Transitional hyaline forms. (4) Polymorphonuclear cells with faintly pink protoplasm, with fine acidophile granules, or with coarse acidophile(eosinophile)granules.
Commentary. The anaemia here is so slight as to be almost unnoticeable. The case is, in fact, one of simple hypertrophy of the spleen associated with rickets.

Case II. J.McL., aet. 10 months. Rickets; anaemia; great enlargement of the spleen and liver.
Blood. 1/4/97. R-C.4,000,000. W-C.19,600. Hb.60.
Proportions of various cells.
Erythroblasts per 100 fields . . . . . -
Mononuclear leucocytes . . . . . .48 per cent.
Transitional leucocytes . . . . . 8 per cent.
Polymorphonuclear leucocytes . . . . .43 per cent.
Eosinophile leucocytes . . . . . 1 per cent.
Microscopic Appearances. (Fig. II.) Red cells are normal. Leucocytes. The only peculiarity is that, while most of the polymorphonuclear cells have an unusually distinct fine acidophile granulation, coarse acidophile (eosinophile)cells are comparatively rare. The leucocytes present are practically the same as those in caseI.
Commentary. The chief point of interest in this case is the occurrence of slight leucocytosis, the relative proportions of the cells remaining about normal.

Case III. H.McG., aet. 13 months. Anaemia; Rickets doubtful; spleen reaches iliac crest.
Blood. 9/3/96. R-C.3,050,000. W-C.15,300. Hb.68.

Figure III. Case IV.

Combined Field: Eosin: methylene blue ×400



a. a' Lymphocytes of varying sizes.
b. basophile mononuclear
c. transitional ~~monocytes~~ basophiles
d.d' polymorphonuclear cells with and without fine acidophil granules
e. megaloblast, showing signs of mitosis

Proportions of the various cells.

Erythroblasts per 100 fields . . . . 17.
Mononuclear leucocytes . . . . . 42 per cent.
Transitional " . . . . . 16 per cent.
Polymorphonuclear " . . . . . 40 per cent.
Eosinophile " . . . . . 2 per cent.

Microscopic Appearances. Red corpuscles as a whole are normal in size and shape. There are a few poikilocytes and normoblasts. Leucocytes. No abnormal cells are present. There appear to be more basophile mononuclear elements than are usually found.

Commentary. In this case we have a simple anaemia with slight leucocytosis. All the varieties of the white cells are increased, especially the transitional forms.

Case IV. L.C., aet. 10 months. Rachitis; anaemia; enlarged spleen and liver.

Blood. 14/4/97. R-C.2,362,000. W-C.14,000. Hb.28.
15/8/97. Under anti-rachitic treatment the spleen has become of normal size; the anaemia has disappeared.

Proportions of various cells. 14/4/97.

Erythroblasts per 100 fields . . . . 6.
Mononuclear leucocytes . . . . . 75 per cent.
Transitional " . . . . . 5 per cent.
Polymorphonuclear " . . . . . 20 per cent.
Eosinophile " . . . . . -

Microscopic Appearances (Fig. III.) Red corpuscles fairly normal; very few nucleated reds found; one of them, however, contained a mitotic figure.
Leucocytes. The majority of these are lymphocytes of various sizes. Most of the larger mononuclear cells contain fine basophile granules; hyaline cells are scanty. The polymorphonuclear leucocytes are normal; some have pale pink bodies, others, fine acidophile granulations. There are no eosinophile cells to be found.

Commentary. In this case we have a simple anaemia with enlargement of the spleen. Considering the age of the child, I do not think there is an evidence of leucocytosis. It will be noticed that the transitional cells are not increased in number. The chief features are (1) the excess of lymphocytes, (2) the numerous basophile leucocytes, and (3) the slightness of the qualitative, as opposed to the quantitative, change in the red corpuscles.

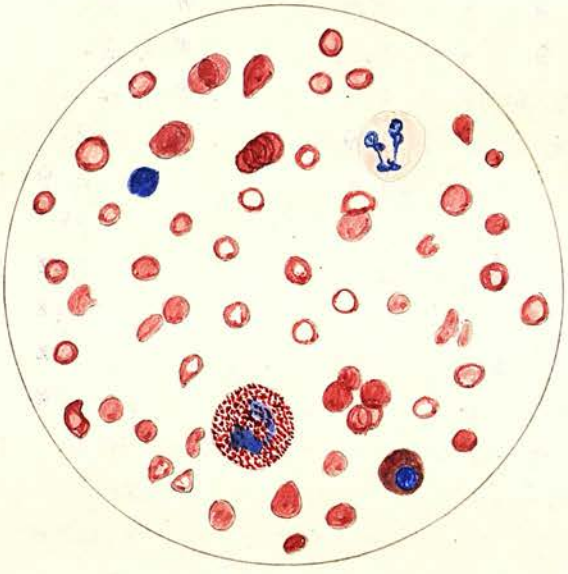
Case V. W.S., aet. 18 months. Diarrhoea and anaemia; rickets doubtful; spleen reaches iliac fossa.
Blood. 15/8/96. R-C.2,600,000. W-C.22,600. Hb.-
2/9/96. R-C.2,900,000 W-C.21,600. Hb.-

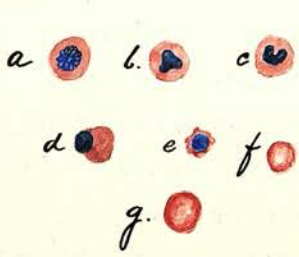
Proportions of various cells	15/8/96.
Erythroblasts per 100 fields	14
Mononuclear leucocytes	52 per cent.
Transitional "	14 per cent.
Polymorphonuclear "	33 per cent.
Eosinophile "	-

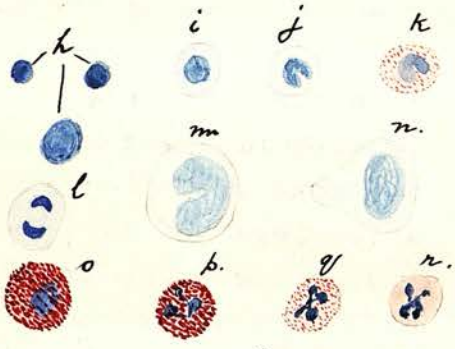
Microscopic Appearances. Red corpuscles shew few qualitative changes; there are some erythroblasts, some of which have double nuclei, and one or two of which are megaloblasts. Apart from the absence of eosinophiles, the appearance of the leucocytes is normal.
Commentary. Here we have a considerable degree of anaemia, but little change in the appearance of the corpuscles. All the forms of white cells, but in particular the transitional type, are increased in number.

Figure IV. Case VI.

Actual Field: Eosin-methylene blue ×700.







a, b. c, d, e. Erythroblasts of different types ~~with~~ variously shaped nuclei: a, shows signs of mitosis.
f. normal red
g. megalocyte.
h. lymphocytes of various types
i hyaline mononuclear
j hyaline transitional
k fine acidophile (neutrophile) transitional
l. mitotic figure leucocyte
m, n, large hyaline mononuclear & transitional
o. Eosinophile myelocyte
p. polymorphonuclear eosinophile
q do. neutrophile
r. do. hyaline.

Case VI. H.C., aet. 16 months. Marked rickets; very great enlargement of the spleen; liver reaches about three inches below the costal margin in the nipple line; considerable anaemia.

Blood. 26/11/95. R-C.3,537,000. W-C.16,300. Hb. -
1/12/95. R-C.3,800,000. W-C.21,800. Hb.60.
4/12/95. R-C.3,500,000. W-C.21,000. Hb. -
29/12/95. R-C.4,400,000. W-C.17,500. Hb.70.
3/3/96. R-C.4,940,000. W-C.12,600. Hb.72.

Proportions of various cells.	1/12/95.	3/3/96.
Erythroblasts per 100 fields	28	a few.
Mononuclear leucocytes	34%	51%
Transitional "	24%	12%
Polymorphonuclear "	35%	37%
Eosinophile "	7%	-

Microscopic Appearances.(Fig. IV.) Red corpuscles. Con-siderable variation in size, in form of central dell, and in shape. The nucleated red corpuscles comprise normo-, micro-, and megalo-blasts, the first-mentioned, however, forming the majority. A few shew signs of division. The protoplasm of the erythroblasts stains deep purplish-brown; the nuclei are well-defined, mostly circular, and stain deeply; they are usually central, but some are eccentric, or project from the cell. In some cases the nucleus is very irregular.

Leucocytes. The small mononuclear(lymphocytes)are normal; the larger mononuclear are of two varieties:- (1) the ordinary form, with clear protoplasm and a nucleus filling a relatively small part of the cell-body (Kanthack's hyaline cells), and (2) very much larger cells of similar structure, measuring from 20 to 30 micros in diameter(marrow cells?). Basophile mononuclear cells are extremely infrequent. In one hyaline leucocyte a distinct mitotic figure was seen.

The transitional types of cells are very similar. Some bear a fine acidophile(neutrophile)granulation.

The polymorphonuclear leucocytes have either a uniform pink body, or shew the neutrophile granulation. In addition to the ordinary eosinophiles, a few mononuclear eosinophiles(eosinophile marrow cells)are present.

On March 3rd, 1896, when the case was again seen, the blood was practically normal and the spleen somewhat diminished in size, the condition being very much what is described in case I.

Commentary. This case, then, is a fairly well-marked example of what was originally described by v. Jaksch — anaemia, enlarged spleen, and leucocytosis. It differs from Luzet's description, however, in the comparative absence of mitoses in the erythroblasts. The leucocytosis is polymorphous, but the transitional cells are relatively more increased than the rest. The chief abnormalities are in the presence of (1) large hyaline mononuclear leucocytes, transitional forms bearing acidophile granules, (3) small eosinophile myelocytes, and (4) the comparative absence of basophile mononuclears.

Case VII. R.P., aet. 10 months. Rickets and laryngismus stridulus; anaemia; spleen greatly enlarged, liver and glands normal.

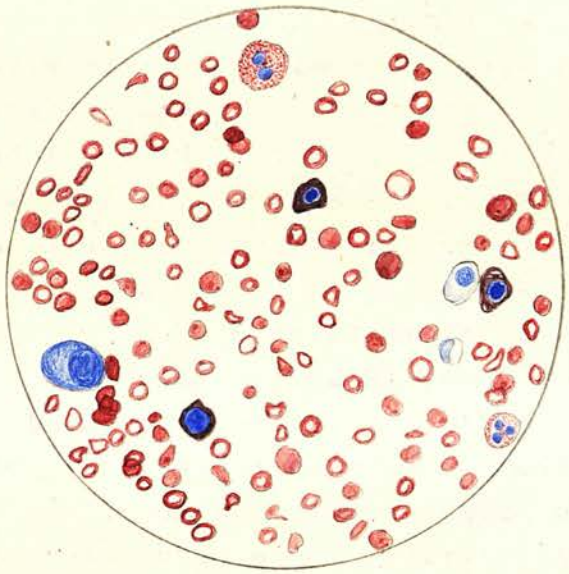
Blood. 27/3/96. R-C. 3,100,000. W-C. 17,500. Hb. 45.
15/1/97. There is no sign of anaemia; the spleen is almost normal in size.

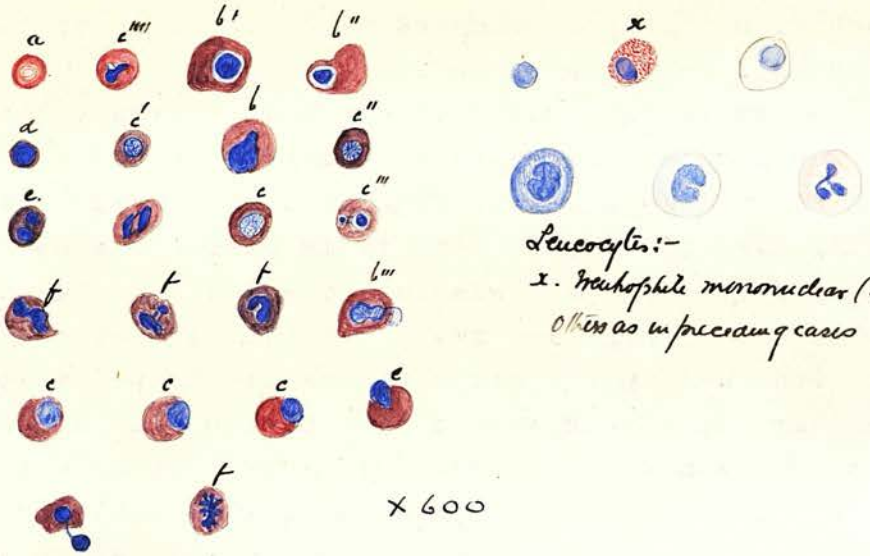
Proportions of various cells.	20/3/96	15/1/97
Erythroblasts per 100 fields . . .	56	2
Mononuclear leucocytes	45%	44·3%
Transitional "	22·5%	7%
Polymorphonuclear "	34%	48%
Eosinophile "	·5%	·7%

Microscopic Appearances (Fig. V.) Red corpuscles. Slight poikilocytosis and variation in size of the central dell. The megalocytes stain uniformly pink. A few cells shew signs of polychromatophilia -- staining purplish-brown like erythroblasts. Among the numerous erythroblasts, considerable variety is seen, as shewn in the figure. Megalo-, micro-, poikilo-, and normo-blasts

Figure V. Case VII.

Actual Field: Eosin methylene Blue ×400





Red corpuscles:-
a, normal erythrocyte
b. megaloblasts
c, normoblasts
d microblast
e. nucleus which has parted
f. fragmentation and irregularity of nucleus
c', c'', show types of mitosis
b', b'' b''', c''', c'''', probably degenerating nuclei

Most cells show polychromatophilia.

Leucocytes:-
x. Neutrophile mononuclear (myelocyte)
Others as in preceding cases

are found, the last-named forming perhaps a third of the total. The protoplasm of the erythroblasts stains either deeply, or of a uniform pink like the normal erythrocyte. As regards the nuclei: the larger forms have a well-marked chromatin network with pinkish material in its interstices; the smaller and more condensed stain deeply and uniformly. In a few cases mitotic figures are seen; in some the nuclei have just divided. Very irregular, branching nuclei are not uncommon, the structure in these cases being apparently in process of fragmentation.

Leucocytes. There is an increase in all the leucocytes, but in particular of the transitional forms.

Of mononuclear leucocytes we have both small (lymphocytes), and larger forms. Some of the latter contain fine basophile granulations, and a few, fine acidophile granulations.

The transitional leucocytes have either a hyaline body, or shew fine basophile granules.

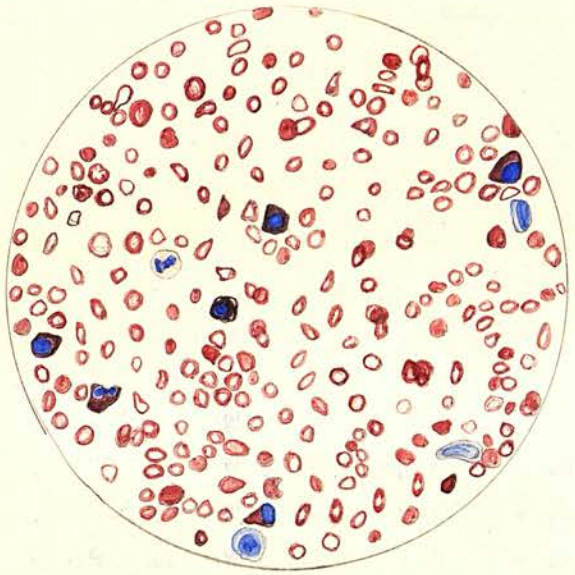
The polymorphonuclear leucocytes are normal; the protoplasm is stained faintly pink with eosin, or shews a more or less abundant fine acidophile granulation.

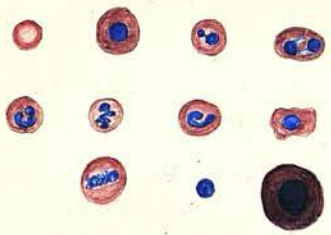
On January 15th, 1897, the films of blood shewed little abnormality. There was less distinction than usual between the large and small mononuclear leucocytes, the fine acidophile granulations of the polymorphonuclear leucocytes were better marked than at the earlier examination, and there were only one or two normoblasts. Subject to these qualifications, the description of the blood of case I. will serve as a description of the blood in this case as a cure resulted.

Commentary. Here we have a great diminution of the red cells and haemoglobin with a great abundance of nucleated red corpuscles, the accompanying leucocytosis being slight. The condition corresponds more closely with Luzet's description than with that of v. Jaksch,

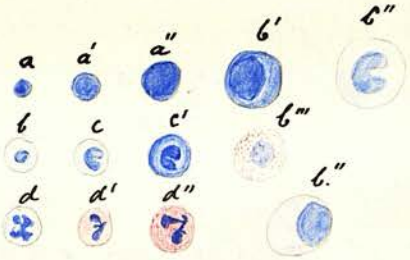
Figure VI. Case VIII.

Actual field: Eosin-methylene blue. Poikilocytosis; numerous erythroblasts shewing marked deformity. X 400





normal red cell, nine nucleated reds, and one free-swimming nucleus. The erythroblasts shew various forms of nucleus & are of different types There is also polychromatophilia



a, a', a"- Lymphocytes
b. hyaline mononuclear
b' large finely basophile mononuclear
b" large hyaline mononuclear
b''' mononuclear with faint neutrophile granulation (myelocyte)
c hyaline transitional
c' basophile transitional
c" large hyaline transitional
d, d' hyaline polymorphonuclear
d" fine acidophile (neutrophile) polymorphonuclear

inasmuch as the leucocytosis, on which the former lays little stress, is not well-marked, while the presence of erythroblasts is a conspicuous feature. The progress towards a complete cure within some ten months, under antirachitic treatment, is an important point in the case.

Case VIII. J.O., aet.11 months. Extreme rickets; spleen very much enlarged, liver slightly enlarged; Very anaemic. Death from bronchopneumonia.

Blood. 17/2/96. R-C.2,640,000. W-C.35,200. Hb.26.
25/2/96. R-C.3,160,000. W-C.27,700. Hb.26.
1/3/96. R-C.3,108,000. W-C.20,000. Hb.30.
9/3/96. R-C.2,600,000. W-C.22,900, Hb.35.

The blood was very pale and watery, and it was very difficult to obtain enough to fill the pipette of the Gowers' haemoglobinometer; the estimations of the haemoglobin, therefore, are possibly less accurate than the enumerations of the cells.

Proportions of the various cells. 25/2/96.

Erythroblasts per 100 fields	47
Mononuclear leucocytes	49 per cent.
Transitional "	24 per cent.
Polymorphonuclear "	26·2 per cent.
Eosinophile "	·8 per cent.

Microscopic Appearances.(Fig. VI.) Red corpuscles. The poikilocytosis was fairly well-marked. On March 2nd it was noted that over fifty per cent of the red cells in freshly-drawn blood were microcytes — small, slightly biconcave, circular, highly refractile structures, of pale yellow colour, and of a diameter about one-quarter or one-third that of a normal erythrocyte. Nucleated red cells are fairly numerous, and comprise normo-, micro-, and megalo-blasts. The nuclei are in most cases central, in some peripheral. Occasionally the nucleus is double, at times it is undergoing fragmentation, and in a few cases mitotic figures are to be seen. Small, pale blue projections can be seen

standing out from some nuclei. The outlines of the erythroblasts are in many cases irregular; sometimes a mere ring of ragged protoplasm surrounds the nucleus. The protoplasm of the cell body stains deeply — sometimes (as in the megaloblasts) so deeply that it is but slightly differentiated from the nucleus. There is a fair number of megalocytes, staining rather darkly, and shewing no central dell.

Leucocytes. The mononuclear leucocytes consist of lymphocytes of various sizes, with deeply staining nuclei, and little or no visible cell body; of hyaline mononuclear cells, large and small; and of finely basophile mononuclears. A few of the cells of this group, although staining of a uniform blue, shewed no signs of granulation. Some mononuclear neutrophiles are present.

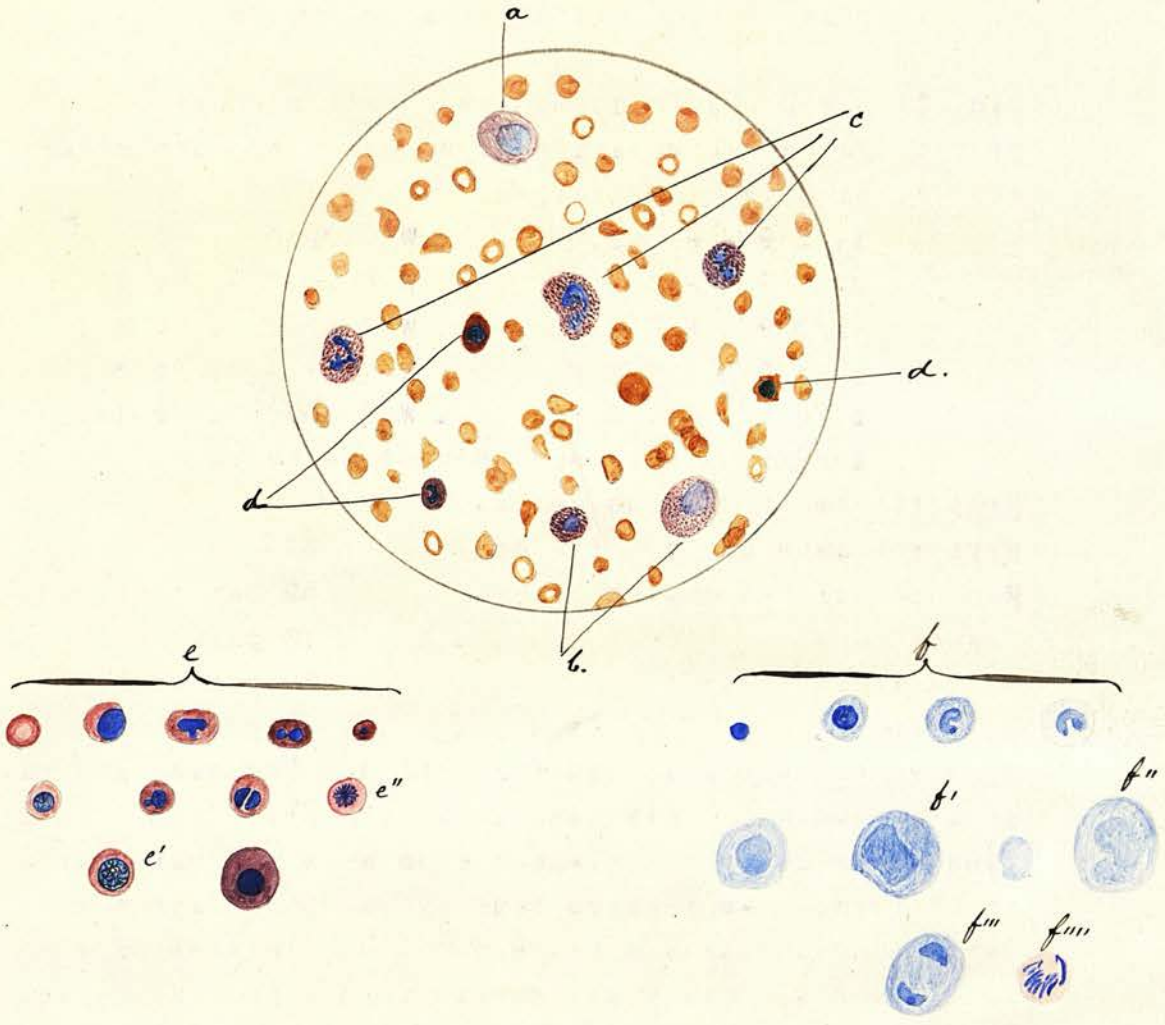
The transitional form of leucocytes was made up of large and small hyaline cells, and those bearing basophile granules.

Some of the polymorphonuclear cells shewed the fine acidophile granules; for the most part, however, the cell body stained uniformly pink, and was free from granulation. In a few case the protoplasm had not taken on the eosin stain at all, but was of a pale blue colour.

Commentary. In this case the condition pretty closely resembles that described by v. Jaksch and Luzet. It is of interest in regard to the etiology of the disease to notice that a twin sister was affected in a precisely similar way. (v. Case IX.) The chief features of the blood as regards the red corpuscles are the poikilocytosis and the presence of erythroblasts. As regards the leucocytes we may note (1) the general absence of the acidophile granulation, and the number of basophile cells present; (2) the presence of neutrophile myelocytes, and of large hyaline mono-

Figure VII. Case IX.

Actual field: Ehrlich Biondi. Shews poikilocytosis, and increase in leucocytes ×700.



a. mononuclear leucocyte
b. do. do , fine acidophile – myelocyte.
e polymorphonuclear do., fine acidophile
d. erythroblast, shewing polychromatophilia
e. do ; e', e", shew signs of mitosis
f. basophile leucocytes. f', f", large fine basophile
f"', divided nucleus, f'''', highly complicated nucleus

Ehrlich Biondi

Eosin-methylene blue

nuclears(myelocytes ?); (3) the blue staining of some of the polymorphonuclear leucocytes; and (4) the relative increase in the transitional elements.

Case IX. E.O., aet. 11 months. Twin sister of the preceding. Rickets; anaemia; spleen very much enlarged, liver slightly enlarged.

Blood. 14/2/96. R-C.2,500,000. W-C.32,500. Hb.-
23/2/96. R-C.2,840,000. W-C.25,700. Hb.27.
2/3/96. R-C.2,920,000. W-C.23,200. Hb.30.
10/4/96. R-C.2,660,000. W-C.18,400. Hb.30.
10/5/96. R-C.2,800,000. W-C.45,000. Hb.18.
16/5/96. Died suddenly of asthenia.

Proportions of various cells.

Erythroblasts per 100 fields	37
Mononuclear leucocytes	52 per cent.
Transitional "	13 per cent.
Polymorphonuclear "	32 per cent.
Eosinophlie "	3 per cent.

Microscopic Appearances.(Fig. VII.) The same general remarks apply to this case as to the preceding. Though less numerous, the nucleated reds shew the same variety of form. As regards leucocytes, there are more large mononuclear and transitional cells shewing a fine basophile granulation than in the preceding case. The polymorphonuclear cells have well-marked fine acidophile granulations, and the eosinophiles are more abundant(3 per cent, as against ·8 per cent). A few marrow cells are present.

Commentary. The condition is practically the same as in the twin sister. In addition to the points of difference mentioned above, it may be noted that the increase in the transitional cells is somewhat less (13 per cent, as opposed to 24 per cent).

Changes in the Blood in Splenic Anaemia.

1. Erythrocytes and Haemoglobin.

When the anaemia is at all well-marked there is usually a relative poverty of haemoglobin. Thus we have Case V. with 2,600,000 red cells, and only 28 per cent of haemoglobin, and Case VII. with over three million red cells and only 45 per cent of haemoglobin, and the same is seen in other instances. This lack of colouring matter is very marked in the severer degrees of anaemia, and is in no way characteristic, being a feature common to most forms of bloodlessness in early life. The amount of haemoglobin present in each corpuscle is fairly well indicated by the staining. In Fig. VII., for instance, most of the red cells are indicated by mere rings — in other words, the central dells have not taken on the stain — while in Figs. I. and II. the corpuscles are, on the whole, more or less uniformly stained throughout.

One feature which has struck me is the comparatively slight degree of poikilocytosis met with even in extreme cases of anaemia. It is most markedly seen in Figs. V. VI. and VII., but, even in these, it is not nearly so striking as in ordinary cases of pernicious anaemia. Irregularity in the form of the central dell — "eine Art der Poikilocytose", according to v. Jaksch — is characteristically seen in Fig. VI., and in the same drawing there is a fair number of oval

corpuscles resembling those figured by Cabot(op. cit.) as occurring in pernicious anaemia. In Cabot's cases, however, all the corpuscles are oval — possibly a reversion to the reptilian type — while here only a comparatively small number shew the deformity.

Polychromatophilia was never observed to any extent so far as the erythrocytes were concerned. The only appearance of it was the somewhat deeper and browner staining of some of the megalocytes. In the case of the erythroblasts, however, polychromatophilia was very evident, and is perhaps illustrated in Fig. V.

The sudden temporary appearance of enormous numbers of microcytes in the blood of one of the patients has been noted. Unfortunately, I have no film shewing the condition.

When we come to consider the relation of the number of erythroblasts to the degree of anaemia(see Table II.), we find that the two are fairly closely allied. But the relationis not constant. Thus, in Case IV., with 2,362,000 erythrocytes, we have only 6 erythroblasts in 100 fields, while in Case VII., with 3,100,000 erythrocytes, we have more than nine times that number of nucleated reds. It might, of course, be suggested that differences in this respect depend on the ages of the children, but, although this is, no doubt, to some extent true, it cannot be the complete explanation; in the two cases just cited, the patients were of the same age. Again, we may find one or two nucleated red

cells in the blood apart from any other sign of anaemia. This is exemplified in the later examinations of Cases VI. and VII. My view is, that the occurrence of a few erythroblasts in such circumstances points to the previous existence of severe anaemia. The erythroblasts do not seem to appear in the blood until the anaemia has reached a somewhat advanced stage: they seem to persist after the regeneration of the red cells and haemoglobin is almost complete.

I have not been able to trace any connection between the severity of the anaemia and the presence of any particular form of erythroblast. Cabot, speaking of pernicious anaemia, considers the presence of even one or two megaloblasts as of much greater prognostic significance than that of a number of normoblasts. In these infantile anaemias, however, the absolute number of the erythroblasts appears to be of greater importance than the presence of any particular type.

In many of the specimens free-swimming nuclei, which had been extruded from the nucleated red cells, were present. These must, I suppose, be regarded as artefacts.

It will be remembered that, in Luzet's opinion, the characteristic feature of the blood in pseudo-leukaemic anaemia is the presence of erythroblasts undergoing mitosis, and it will no doubt be said that my drawings do not shew this to any marked extent. I have found

mitotic figures in some of the cells in the severer cases, but they are not so numerous as one would expect from reading Luzet's paper. When we come to look at his plates, however, it will be found that the cells he depicts(op. cit., Planche II. figs. 17 - 33)are very much the same as those of my cases. In fact, what appears to be really a degenerative process — a fragmentation — in the nucleus, is described by Luzet as a mitotic figure. The point on which I would insist is that the condition described and figured by him, is identical with that found in some of my cases.

The relationship between the extent to which a leucocytosis takes place and the degree of the anaemia (Table I.)is not more precise than the ratio of the nucleated to the non-nucleated red cells. In general, the two — leucocytosis and diminution of red corpuscles — run together, but exceptions occur. Thus, in Case IV. we have marked anaemia, but a number of white cells but slightly above normal, while in Case II. the converse occurs. In the latter, however, I cannot exclude the possibility of a digestive leucocytosis; the child was living at home and getting food at irregular intervals. The fact that the normal proportion of the different forms of cells was preserved seems to favour this view.

2. Leucocytes.

I have never met with any cases in which the number of the white cells was so high as that recorded by v. Jaksch — 60,000 to 80,000 or more per cmm. — but he seems to have observed a more marked leucocytosis than most other investigators.

Normally, as in case I., we find (a) lymphocytes of different sizes; (b) hyaline cells with round, oval, or indented(transitional)nuclei; (c) a few mononuclear cells whose protoplasm stains deeply with methylene blue, and apparently contains fine basophile granules; (d) polymorphonuclear cells with either a pale pink protoplasmic body, or one containing fine acidophile (more conveniently, neutrophile)granules or eosinophile granules.

The leucocytosis of the cases we are considering is essentially polymorphous, and varies somewhat in the appearance it presents. So far as the proportions of the various cells go, its most striking characteristic is that there is always a relative increase in the transitional type of cell(Table I.). Thus we find such figures as 16 per cent, 22 per cent, and 24 per cent, in place of the normal — 7 per cent. And in the later examinations of Cases VI and VII, which had recovered from the disease, we find that the number of these cells has fallen from 24 and 22·5 per cent, to 12 and 7 per cent respectively. I cannot but look upon this increase in the transitional type of leucocyte as some-

what characteristic of the condition we are discussing, but I have not been able to find any specific reference to it in the literature of the subject.

With regard to the staining reactions of the leucocytes, it will be noted that in Case VI. a few marrow cells, both eosinophile and neutrophile, were present. In Cases VII, VIII, and IX, the former variety was also noticed. In addition to these typical granular myelocytes, very large cells with hyaline bodies and faintly staining nuclei(hyaline marrow cells?)were found in Case VI. The marrow cells, of whatever kind, were never at all numerous, and required to be carefully sought.

Another feature of the leucocytosis, which I think is rather characteristic, is the occurrence of considerable numbers of mononuclear leucocytes, whose protoplasm stains deeply with methylene blue and has a finely granular appearance. These appear to be basophile cells, and they were present in most of the cases(III, IV, VII, VIII, and IX)in considerable abundance. In the worst cases too, some of the transitional leucocytes had a similar appearance, and there could also be found some large basophile elements, having a diameter of perhaps 20 or 30 micros, which are certainly not normally met with.

The polymorphonuclear leucocytes shewed little deviation from the normal. In one case the distinction between the fine and the coarse acidophile granules was slight. This is probably not very unusual, since, as

Gulland (39) has shewn, the distinction between strongly and slightly eosinophile granules is purely arbitrary.

The number of eosinophile cells varied greatly, and does not appear to be of any clinical significance.

To summarize the above: in marked cases of anaemia with splenic enlargement we may have:-

(1) Diminution of erythrocytes and haemoglobin.

(2) Considerable variation in the sizes of the corpuscles, their shapes being relatively well preserved, but the appearance of the central dell being very often altered.

(3) The presence of numerous erythroblasts of all forms: their nuclei may be of irregular outline, may be undergoing fragmentation, or may shew mitotic figures.

(4) The presence of free-swimming nuclei.

(5) Leucocytosis in which all forms of cell participate, but in which especially the transitional type is increased. The relative increase of this variety takes place at the expense of the polymorphonuclear, and, to a less extent, of the mononuclear forms.

(6) The presence of abnormal leucocytes, such as (a)large hyaline(marrow?)cells; (b)eosinophile, and (c)neutrophile marrow cells; and (d)large

finely basophile cells.

(7) An excessive number of finely basophile cells of ordinary size.

Pathology.

I have few remarks to offer as to the pathology of the disease: substantially, I agree with Luzet's view that it is a return to the foetal condition, although in my only post mortem(Case VIII.)the liver did not shew the lesion described as characteristic by that writer.

If we assume that the so-called transitional cells are physiologically, as well as morphologically, intermediate between the mononuclear and the polymorphonuclear leucocyte, we may suppose that the leucocytosis is due as much to a retardation in the evolution and decay of the leucocytes, as to an increased production of them. It is a matter of every-day knowledge that leucocytosis is an extremely common accompaniment of all secondary anaemias of childhood; indeed, Monti and Berggrun(40) propose to classify anaemias on the basis of the presence or absence of leucocytosis.

With regard to the relation of the enlarged spleen to the condition of the blood, I have elsewhere (op. cit.)expressed the opinion that both are secondary to some other disease — almost invariably rickets, occasionally syphilis. The anaemia is either directly due to the primary disease, or may possibly occur as a more or less accidental concomitant. But, and this seems to

me the important point, the condition of the blood may be profoundly modified by the enlargement of the spleen. The severity of rachitic anaemia is, as Morse(41)points out, proportionate to the degree of the osseous lesion, but no definite blood change is associated with rickets. In the series of cases observed by Morse, leucocytosis was chiefly found where there was splenic enlargement. Starck(42)thinks that the splenic tumour in rickets depends on the anaemia, and Ashby and Wright(43)state that when severe anaemia follows rickets the spleen is often enlarged. Notwithstanding the work of Dickinson (44), who regards a fibrous overgrowth of the spleen as pathognomonic of rickets, of Kuttner(45), of Cohn(46), of Fox and Bell(47), and of many others, we are still in the dark as to the exact connection between enlargement of the spleen and rickets and syphilis. There seems, however, little doubt that it is directly due to these maladies, and not merely accidental. As it occurs independently of anaemia(in many cases of simple splenic hypertrophy there is neither sign nor history of anaemia) it seems only logical to suppose that when anaemia of this particular type — never found apart from splenic hypertrophy — develops, it in all probability owes its characteristic features to that hypertrophy.

Table III

Group	Case	Date	Red Corpuscles	Leucocytes	Haem.	Erythroblasts p. 100 fields	Percentage of			
							Mononuclear	Transitional	Polynuclear	Eosinophile
I	VII	15/1/97	—	—	—	2	44·3	7	48	·7
II	I	15/1/97	4,900,000	10,500	80	—	55	7	37	1
	VI	3/3/96	4,900,000	12,600	72	a few.	57	12	37	—
III	IV	14/4/97	2,362,000	14,000	28	6	75	5	20	·
IV	II	11/4/97	4,000,000	19600	60	—	48	8	43	1
	III	9/3/96	3,050,000	15,300	68	17	42	16	40	2
	V	15/8/96	2,600,000	22,600	—	14	52	14	33	—
V	VI	11/12/95	3,800,000	21,800	60	28	34	24	35	7
	VII	20/3/96	3,100,000	17,500	45	56	45	22·5	34	·5
	VIII	17/2/96	2,640,000	35,200	26	47	49	24	26·2	·8
	IX	16/4/96	2,800,000	45,000	18	37	52	13	32	3

Clinical Aspects of the Matter. (Table III.).

In Table III. I have arranged the cases in five groups. It will be noticed the each of the cases observed during convalescence occurs in two of the groupps.

The Case in group I. may be called one of rickets with slight enlargement of the spleen. The patient shewed no sign of anaemia.

In group II we have what is to be regarded as rickets with splenic hypertrophy. Such cases are not uncommon.

In group III. we have a fairly well-marked example of a condition very similar to the splenic anaemia of adults — but associated with rickets.

In group IV. we have a series of intermediate cases; according to Monti and Berggrün's classification the first would be "anaemia levis cum leucocytosi", the second and third, "anaemia gravis cum leucocytosi".

Group V. consists of cases of so-called pseudo-leukaemic anaemia.

But this division is entirely arbitrary. Intermediate cases, which it is impossible to bring absolutely into any one of these groups, constantly occur. It seems to me that we should, for the present at least, group all these cases as secondary anaemias, associated with splenic enlargement, usually of rachitic origin.

At the risk of unnecessary repetition, I may recapitulate the grounds on which the conclusion is based:-

(1) In almost all reported cases of pseudo-leucaemic anaemia the patients have been rachitic; in a few, syphilitic. The two most extreme cases which have come under my notice occurred in rachitic twins.

(2) Marked enlargement of the spleen from simple hypertrophy occurs in a small proportion of cases of rickets and congenital syphilis; it does not — in children at least — appear to occur apart from these diseases.

(3) The characteristic condition of the blood described above only occurs when a large splenic tumour is present.

(4) Many intermediate stages may be observed between this typical condition and one of simple anaemia.

(5) Antirachitic treatment is by far the most serviceable in bringing about a cure of the condition.

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